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# Interactive Effect of Manganese, Molybdenum, Nickel, Copper I and II, and Vanadium on the Freshwater Alga *Scenedesmus quadricauda*

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Toxicity testing is a very important issue in all communities including scientific, commercial, ecological and regulatory bodies. The combination of increased industrialization with the concomitantly increased demand for chemicals, in both developed and developing nations, has led to increasing ecological and toxicological problems due to the release of toxic contaminants into the environment. Toxic impact of chemicals on humans or environment has usually been tested only on the basis of a single chemical, although exposure to a single chemical is not the most prevalent situation. Simultaneous or sequential exposures to two or more chemicals can change the toxicity of each chemical both quantitatively and qualitatively (Wang et al. 1995; Tomasik et al. 1995). The same is valid for physical and chemical properties of single chemicals used in predictive models. There have been many attempts to predict the joint toxic effects of mixtures of chemicals (Skipnes et al. 1975; Braek et al. 1976; Stromgren 1980; Verriepoulos et al. 1987; Bennet and Arthur 1989; Wu 1991; Tomasik et al. 1995; Wang et al. 1995; Tichý et al. 1999). Individual substances, which are toxic to organisms when acting individually, often produce unexpected effects when combined with others. One toxin may affect other toxins by interfering in reaction steps involving them (Verriepoulos et al. 1987). Thus interactive (reciprocal, mutual, bilateral) chemical reactions between substances may also affect the toxicity. In practice, various pollutants often coexist in one system. The interactive effect of several toxins is an important factor when evaluating their actual toxicity in the environment (Wang et al. 1995; Tomasik et al. 1995). The combined effect of multi-pollutants is very complicated. It is related not only to the composition of the mixture, but also to the organisms that the toxin acts upon (Tomasik et al. 1995). The effect in general is confined to the result of a study of individual metal toxicity. Because of many paradoxes a set of complete theories on the mechanism of the effect of individual metal ions on various aqueous organisms has so far not been established (Wang et al. 1995). Stromgren (1980), Bennett and Arthur (1989) and Wang et al. (1995) carried out studies on interactive (mutual) effects of various metals on algae. However, conclusions were inconsistent and currently no common view exists.

Intensive studies on various models resulted in numerous reports and some reviews (Chang and Sibley 1993; Tomasik et al. 1995). Dirilgen and Inel (1994) and Lee et al. (1994) attempted to quantify predictions of the toxicity resulting from metal-metal interactions in several biological systems. Interaction is a process by which metals in their various forms change the critical concentration or a critical effect of the metal under consideration (Nordberg 1976). Such changes are regarded as interactions only if they deviate from single additivity. In other words, interactions should be called positive, if they cause over-additive (synergistic) effects, and negative, if they cause less than additive (antagonistic) effects. The reciprocal effect is depending on the metal form (complexes, chelates etc.), algae species, metal-metal concentration ratio and environmental conditions (Wang et al. 1995).

This paper studies the interactive effects of V, Ni, Mo, Cu(I), Cu(II) and Mn on freshwater green alga *Scenedesmus quadricauda* and presents a preliminarily interpretation of the individual metals and their combined pair toxicity.

## MATERIALS AND METHODS

$V_2O_5$; $NiSO_4.7H_2O$; $MnSO_4.H_2O$; $(NH_4)_6Mo_7O_{24}.4H_2O$; $CuSO_4.5H_2O$ and $Cu_2Cl_2$ were taken as the source of metal ions. All compounds were of analytical grade p.a. (MERCK, Darmstadt, Germany). Manganese, Mo, Ni and Cu were chosen since they are known to be essential trace elements up to a certain concentration, above which inhibitory or lethal effects might occur. Vanadium (possibly essential) was chosen because it is, together with Ni, present in fossil fuels and is concentrated in the ash and emissions when these fuels are burned. Unfortunately vanadium is often not distinguished in the literature (Merian 1991). The following binary metal combinations were used: V+Ni; V+Mo; V+Cu(II); V+Cu(I); V+Mn; Ni+Mo; Ni+Cu(II); Ni+Cu(I); Ni+Mn; Mo+Cu(II); Mn+Cu(I); Mo+Mn; Mn+Cu(II); Mn+Cu(I). For single and binary combination tests, metals ions were applied as solutions with the following concentrations (mg/mL): V – 2.25; Ni – 0.6; Mo – 2.5; Mn – 5.2; Cu(I) – 0.30; Cu(II) – 0.26. These concentrations were equal to $EC_{50}$ values for algae growth inhibition and were already calculated by Fargašová et al. (1999). All experiments were set up in a completely randomized design with 3 replications. Results were evaluated using statistical software QC.Expert$^{TM}$ (TriloByte, Ltd., Czech Republic). The 5% alpha level was used in all statistical tests.

*Scenedesmus quadricauda* (TURP.) BRÉB., strain GREIFSWALD/15, was grown in a liquid culture of modified Knop solution without calcium (Fargašová 1994). The culture was maintained under constant temperature (25±1 °C) and permanent light conditions (24 $\mu Es^{-2}m^{-2}$). The algae were statically cultivated during the tests in 100-mL Erlenmeyer flasks with 50 mL cultivation medium supplemented with individual metals and selected combinations. The suspension density at the beginning of all experiments was about 25,000 coenobia (four cells connected in one unit) per 50 mL in the test and control media. Algae were in the exponential phase of the growth. The experiments lasted 12 days. The growing rates of the algae were expressed by the optical density of the cultural medium at $\lambda$=750 nm (Bolier and Donze 1989). The content of chlorophyll a and the total chlorophyll content were determined by using a spectrophotometric method (Fargašová 1999). Chlorophyll content was determined in a 95% ethanol extract measuring absorbance at 665 and 649 nm, respectively. It was then calculated by the following equations:

$$\text{chlorophyll } \underline{a} \text{ (chl}\underline{a}) = 13.70(A_{665}) - 5.76(A_{649})$$
$$\text{total chlorophyll (chlt)} = 6.1(A_{665}) + 20.04(A_{649})$$

The chlorophyll content being expressed in µg/mL.

The interactive effect of metals on algae *S. quadricauda* was calculated with the equations introduced by Wang et al. (1995). The cellular growth equation for algae is:

$$dN/N_0 = r^* dt$$

where $N_0$ is the cellular density; $r$ is the breeding potential of the control algae; $t$ is time of cultivation. If the toxin is added, the equation changes to:

$$dN/N_0 = (r - R)\, dt$$

where $R$ is the efficiency of a toxin in retarding the growth of algae. Combining the above two equations, we obtain:

$$R = -\ln(N/N_0)/t$$

The greater the value of $R$, the more toxic the toxin is. With two metals of $R$ values $R_1$ and $R_2$, the collusive effect can be either:

$$R_{1+2} \quad \begin{array}{ll} > R_1 + R_2 & \text{synergism} \\ = R_1 + R_2 & \text{additivity or} \\ < R_1 + R_2 & \text{antagonism} \end{array}$$

The degree of antagonism of two metals depends on the value of $A$, where

$$A\ (\%) = 100(R_1 + R_2)/\ R_{1+2}$$

If $A$ <1 synergism occurs, $A$ >1 antagonism occurs. The larger the $A$-value, the stronger the antagonism.

The same equations were also used for determination of metal reciprocal effect on photosynthetic pigment production.

## RESULTS AND DISCUSSION

In the first part of the experiments, we have tried to show how severely metal ions were able to alter the growth and the chlorophyll content of freshwater alga *Scenedesmus quadricauda.* The efficiency values ($R$) for individual metals tested in $EC_{50}$ concentrations and measured values for observed parameters are shown in Table 1. The efficiency values were calculated with the equations published by Wang et al. (1995) and they present nominal values. Under the assumption that the greater the value of $R$ the more toxic is the metal, one can conclude that Ni and Mo were the most toxic to the alga growth. There were no significant differences in the efficiency of other metals tested (V, Cu(I), Cu(II), Mn) to inhibit the growth of *S. quadricauda*.

**Table 1.** Evaluation of *S. quadricauda* alga density, total chlorophyll and chlorophyll <u>a</u> content after 12 days of cultivation in the presence of individual metals and calculated efficiency values (*R*) for the observed parameters.

| Metal | $EC_{50}$ conc. (mg/L) | Coenobia/mL ± SD | Total chlorophyll ± SD (μg/mL) | Chlorophyll <u>a</u> ± SD (μg/mL) |
|---|---|---|---|---|
| V | 2.25 | 2 395 833±180 422 (0.063) | 0.164±0.049 (0.051) | 0.101±0.019 (0.060) |
| Ni | 0.6 | 2 187 500±156 250 (0.071) | 0.146±0.025 (0.060) | 0.087±0.004 (0.008) |
| Mo | 2.5 | 2 239 583±180 422 (0.069) | 0.169±0.010 (0.048) | 0.094±0.026 (0.066) |
| Mn | 5.2 | 2 447 917±90 211 (0.061) | 0.192±0.010 (0.037) | 0.133±0.018 (0.037) |
| Cu(I) | 0.30 | 2 552 083±90 211 (0.058) | 0.197±0.035 (0.035) | 0.161±0.045 (0.021) |
| Cu(II) | 0.26 | 2 500 000±156 250 (0.059) | 0.199±0.031 (0.034) | 0.158±0.049 (0.023) |
| Control | | 5 104 167±238 676 | 0.301±0.021 | 0.207±0.010 |

SD – standard deviation; ( ) values in the brackets means the efficiency values *R* of metals

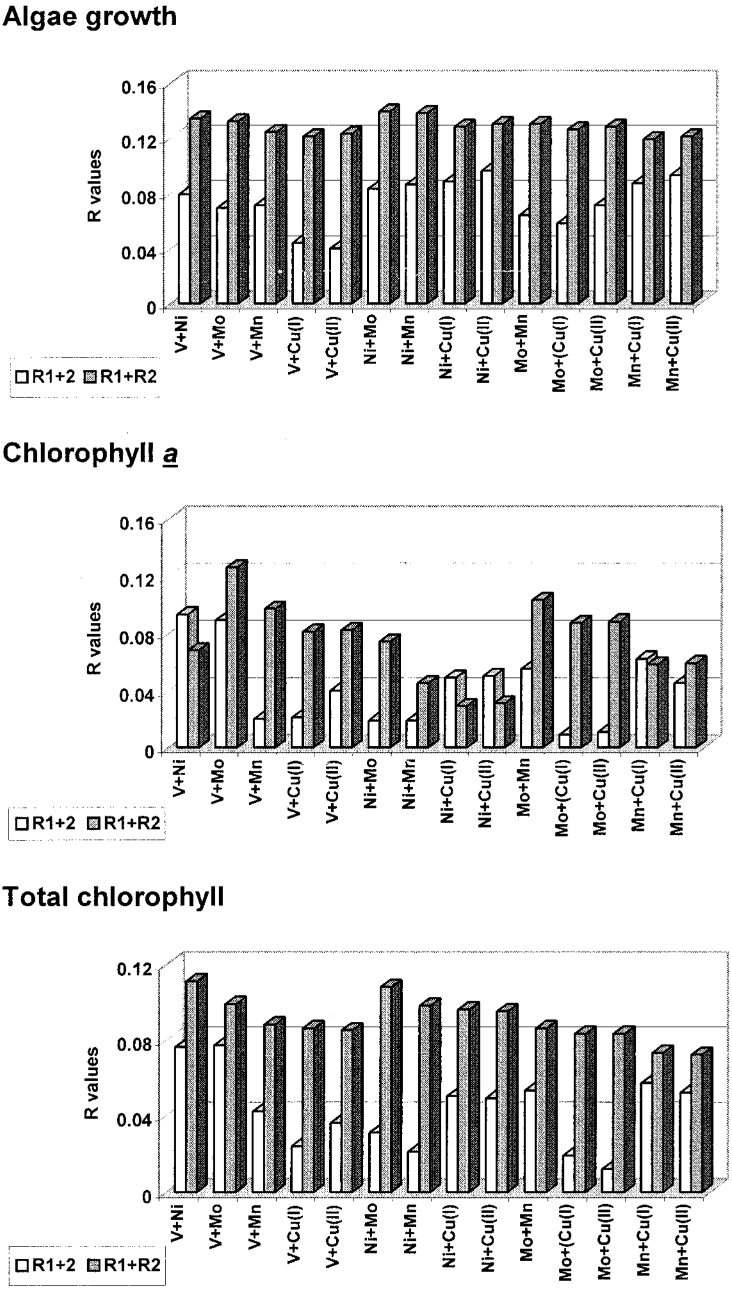
Recently short-term effects of toxicants on rates of physiological processes were be employed as an alternative to the standard alga bioassay methods. Photosynthesis and photosynthetic pigment production are more sensitive to low concentrations of some toxicants than population growth (Giddings et al. 1983). Since chlorophyll <u>a</u> comprises 1 to 2% of the dry weight of most alga cells and since it is easily quantified spectrophotometrically, this photosynthetic pigment has been widely used for the determination of phyto-plankton and periphyton biomass (APHA 1985). The calculated nominal *R*-values (Tab. 1.) for both chlorophyll contents were generally many times lower than those for alga suspension density. This indicates a weaker negative effect of metals tested on the production of photosynthetic pigment than on the growth of alga *S. quadricauda*. The same conclusions were also drawn for some heavy metals (Cd, Pb, Zn, Cu, Fe) (Fargašová 1999). The highest retardation in chlorophyll production was observed for V and Mo ions. The calculated *R*-values for efficiency were for chlorophyll <u>a</u> not much different from those for alga growth. Low efficiency on pigment content was observed mainly for Cu in both oxidation states. In the literature attention has been concentrated especially on the effect of copper (Tubbing et al. 1994) or manganese, zinc and nickel (Lee et al., 1994). Fathy's and Falkner's (1997) data show that copper toxicity for algae depends on the environmental levels of copper. Copper has in all scientific reports (Ivorra et al. 1995) been classified as being extremely toxic for most photosynthetic aqueous organisms and this statement is in agreement with the results of this paper.

**Table 2.** Evaluation of the density of *S. quadricauda* alga, total chlorophyll and chlorophyll *a* content after 12 days cultivation in the presence of metal combinations

| Metal combination | Coenobia/mL ± SD | Total chlorophyll ± SD (μg/mL) | Chlorophyll *a* ± SD (μg/mL) |
|---|---|---|---|
| V + Ni | 1 979 167±90 211 | 0.121±0.004 | 0.068±0.008 |
| V + Mo | 2 239 583±180 422 | 0.119±0.008 | 0.071±0.018 |
| V + Mn | 2 187 500±156 250 | 0.182±0.018 | 0.162±0.025 |
| V + Cu(I) | 3 020 833±90 211 | 0.225±0.014 | 0.160±0.013 |
| V + Cu(II) | 3 177 083±90 211 | 0.196±0.011 | 0.128±0.015 |
| Ni + Mo | 1 875 000±156 250 | 0.208±0.014 | 0.164±0.013 |
| Ni + Mn | 1 822 917±238 676 | 0.235±0.002 | 0.164±0.026 |
| Ni + Cu(I) | 1 770 833±90 211 | 0.166±0.025 | 0.115±0.055 |
| Ni + Cu(II) | 1 614 583±90 211 | 0.168±0.014 | 0.113±0.014 |
| Mo + Mn | 2 369 792±162 630 | 0.156±0.001 | 0.107±0.004 |
| Mo + Cu(I) | 2 552 083±90 211 | 0.239±0.038 | 0.185±0.030 |
| Mo + Cu(II) | 2 187 500±156 250 | 0.261±0.024 | 0.182±0.023 |
| Mn + Cu(I) | 1 787 500±156 250 | 0.152±0.014 | 0.098±0.013 |
| Mn + Cu(II) | 1 666 667±90 211 | 0.161±0.005 | 0.120±0.012 |
| Control | 5 104 167±238 676 | 0.301±0.021 | 0.207±0.010 |

SD – standard deviation

The interactive (reciprocal, mutual) toxic effects of binary metal combinations expressed for alga growth and chlorophyll's production are presented in Figure 1. The measured values of observed parameters were used for calculation of the nominal efficiency values by equations reported by Wang et al. (1995), and are shown in Table 1 and Table 2. The efficiencies of metal-metal combinations demonstrate that the reciprocal effect of metals tested (V, Ni, Mo, Cu(I), Cu(II), Mn) is in our experimental range for all observed parameters mainly antagonistic. The interactive effect in all tested binary combinations for alga growth was always antagonistic. This indicates a better alga growth in the presence of metal combinations than in the presence of individual metals. For the production of chlorophyll reciprocal interactions depend on the observed type of chlorophyll (Fig. 1.). While for total chlorophyll in tested combinations only antagonistic effect was determined, for chlorophyll *a* in combinations V+Ni, Ni+Cu(I), Ni+Cu(II) and Mn+Cu(I) a synergism was observed.




**$R_{1+2}>R_1+R_2$ synergism; $R_{1+2}=R_1+R_2$ additivity; $R_{1+2}<R_1+R_2$ antagonism**

**Figure 1.** Efficiency values (R) versus metal-metal combinations with regard to algae growth and chlorophyll content.

Very little information can be found on the subject *S. quadricauda* and the tested metal-metal combination effects on the mentioned physiological processes. In the literature most of the interest is focused on the metal pair combinations with zinc. Zinc is usually introduced as an antagonist to many metals and thus rapidly decreases their toxicity (Lee et al. 1994). However, interaction between zinc and other metal in combination, for example Cd or Cu, expressed through calculated *R* values depends on the ratios of both metals as well as on the used alga species (Wang et al. 1995). During our tests most metal-metal combinations reduced the unfavorable effect of metals on alga growth as well as on total chlorophyll and chlorophyll *a* production. This indicates an antagonistic effect between the metals tested. Some exceptions were found only in the combinations V+Ni, Ni+Cu(I), Ni+Cu(II) and Mn+Cu(I) when we observed a reduction in the chlorophyll *a* content.

The data on the toxicity mechanism of metals (both individual and in pair combinations) to phytoplankton are insufficient to allow firm conclusions (Wang et al. 1995). The study of inhibition of some physiological and enzymatic activities while metals enter the cells are very important. The present study has tried to give some indication for mathematical interpretation of interactions in binary metal combination for freshwater phytoplankton. The type of metal-metal interaction can be determined bilaterally by comparison of the calculated efficiency values for individual metals and their pair combinations.

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