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# Correlation between Heavy Metal Ions (Copper, Zinc, Lead) Concentrations and Root Length of *Allium cepa L.* in Polluted River Water

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## ABSTRACT

*The present work was performed using the common onion (*Allium cepa L.*) as a bioindicator of toxicity of heavy metals in river water. The test waters were collected at two sampling sites: at the beginning and the end of the Toledo River. The bulbs of* A. cepa L. *were grown in test water with nine concentration levels of copper, zinc and lead from 0.1 to 50 ppm. In the laboratory, the influence of these test liquids on the root growth was examined during five days. For test liquids containing below 0.03-ppm dissolved Cu the root growth was reduced by 40% However, the same reduction occurred for 1-ppm dissolved Zn. For dissolved Pb, results reveal toxicity above 0.1 and 0.6 ppm at the beginning and the end of the Toledo river water, respectively.*

**Key words:** Acute toxicity, bioassay, copper, zinc, lead, *Allium cepa L*

## INTRODUCTION

In 1938 the use of *Allium cepa* was introduced as a biological test system to evaluate the cytogenetic effects of colchicine cells (Levan, 1938). Since then, *A. cepa L* (common onion) has been a biological material of wide use in laboratory tests, due to the fast growth of its roots and the response of genetic material to the presence of potential cytotoxic and genotoxic substances in test liquids (Vesna et al., 1996). The *Allium* test has been applied to evaluate the quality of underground, surface waters and effluents in a simple way through the study of macroscopic parameters, such as the values for root growth inhibition, cytological parameters such as aberrations cellular metaphase and anaphase and cellular division inhibition (Vesna et al., 1996 and Fiskesjö, 1988).
In the last decades, the pollution level or river water quality has been determined by physical/chemical tests, saprobiological, radiological, cytogenetical and genotoxical analysis in *Allium cepa L.* (Vesna et al., 1996). Inhibition of root length is suitable for evaluation of substances in various concentrations. The simplicity of the *Allium* test procedure is the reason for its recommendation by international environmental protection agencies for estimating

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environmental pollution and toxicity caused by industrial effluents, non-treated municipal or domestic waste water.

The aim of this paper was to estimate the *A. cepa L.* sensitivity for different heavy metal ions and demonstrate the effects of pollution on onions, and to carry out environmental education projects, so that it could be used as a test organism for toxicity assessment in polluted river water or otherwise.

## MATERIALS AND METHODS

*The test organism*

Commercial variety bulbs of the common onion (*Allium cepa L.*) were purchased locally. Only bulbs in good condition and of approximately 3.5 cm diameter were used. The bulbs were cleaned by cutting off their dried roots, washed, dried and then stored in a refrigerator at $4^oC$.

*Test liquids*

The experiments were carried out using water from two different sampling sites of the Toledo River. The Toledo basin is located in the Brazilian state of Parana between latitudes of 24°46´ and 24°45´ S and longitudes of 53°34´ and 53°46´ W and drains an area of approximately 97 $km^2$ over an extension of 27 km.

The first sampling site (Clean Water - CW) was located at the beginning of the river where, according to the Brazilian environmental legislation (CONAMA 20, 1986), the water pollution level has been classified as II. The second sampling site (Polluted Water -PW) was downstream the Toledo city. There, the water received the addition of partially untreated urban and industrial effluents and was classified as III.

A set of 1000-$mgL^{-1}$ standard solutions was prepared for Cu, Zn and Pb using their metal salts ($CuSO_4$, $Pb(NO_3)_2$ and $ZnSO_4$) dissolved in de-ionized water and stored in acid-washed volumetric flasks. To obtain the test liquids loaded with metallic ions, regular river water from both the sampling sites was mixed with the standard solutions, diluted in nine treatment concentrations, ranging from 0.1 to 50 $mgL^{-1}$. As a control, normal river water was used.

*Test procedure*

A set of 200-ml polyethylene cups and their respective 5.0-cm diameter lid, washed with diluted nitric acid (10%) were used. To fix each bulb in the central and upper part of the cup and directly in contact with the liquid, the lids were perforated with a 3.0-cm hole. Each onion bulb was placed in a 200-ml polyethylene cups filled with the test or control water and firmly fixed on the perforated lid. For both water types (CW and PW), the three elements, and the 10 test solutions, a total of 600 bulbs were grown for 5 days in the laboratory.

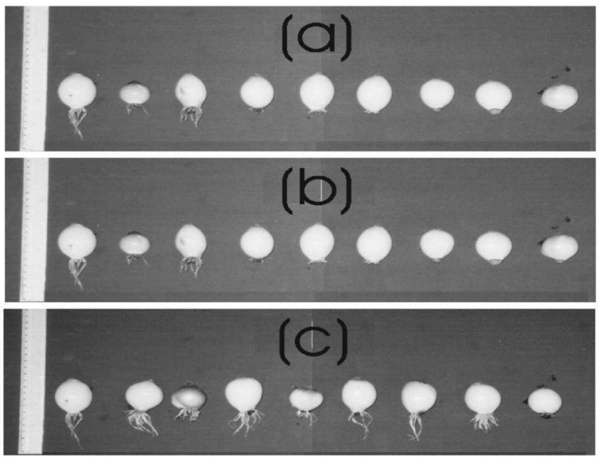


**Figure 1 -** Examples of macroscopic effects on *Allium cepa L.* roots after five days of growth in polluted river water enriched with increasing metallic salt concentrations (a) $CuSO_4$, (b) $ZnSO_4$ , and (c) $Pb(NO_3)_2$. Metallic concentrations range from 0.0 (on the left) to 50 $mg.L^{-1}$ (on the right).

The tests were performed at room temperature (about 20°C), with a natural light-dark regime, and protected against direct sunlight. After growing, test and control waters were collected for dissolved concentrations analysis, and the mean length values in the root bundles measurement. Some selected test series are shown in Fig. 1.

*Concentration measurements by TXRF*

The amount of dissolved metals contained in the test and the control water were measured using the Total Reflection X-ray Fluorescence, TXRF. These measurements were done to verify the real amount of heavy metals in solutions. For TXRF analysis, a 60 mL aliquot of each test water (CW and PW) was separated and filtered using a Millipore filtration system with a 47-mm diameter cellulose acetate membrane with 0.45-µm pore size. After filtration, a 10 mL aliquot of each sample was spiked with 10 µL of the yttrium stock solution (11.6 g Y/l), as an internal standard. Aliquots of 5 µL were deposited on pre-cleaned acrylic disks (ϕ 32mm, 3mm thick) and were dried at room temperature. For TXRF analysis, the samples with the dry residues were irradiated with a polychromatic X-Ray beam with maximum energy of 20 keV, at the Brazilian Synchrotron Light Laboratory (LNLS), in Campinas, São Paulo. The X-ray spectra were analyzed using the AXIL program. Using the calibration curve for the TXRF system, the dissolved metallic concentrations were determined.

## RESULTS AND DISCUSSION

The concentrations of dissolved metals obtained by the TXRF system were very different from the initial concentration of the same metals added to both river water (CW and PW), as shown in the Table 1. During the experiment, some of these added metallic salts could have formed chemical complexes and precipitated, reducing the amount of dissolved metals available for the change of root growth. The concentration measurements for copper and zinc in control water showed very low values, while for lead these were below the detection limits (1 ppb) for TXRF technique.

**Table 1 -** Total and dissolved concentrations of Cu, Zn and Pb in the test waters (CW and PW). The relative uncertainty <15%.

| Total Conc. (ppm) | Cu dissolved (ppm) | | Zn dissolved (ppm) | | Pb dissolved (ppm) | |
|---|---|---|---|---|---|---|
| | CW | PW | CW | PW | CW | PW |
| 0 | 0.012 | 0.020 | 0.35 | 0.10 | 0 | 0 |
| 0.1 | 0.022 | 0.067 | 0.17 | 0.17 | 0.032 | 0.011 |
| 0.3 | 0.073 | 0.092 | 0.25 | 0.35 | 0.077 | 0.061 |
| 0.5 | 0.11 | 0.19 | 0.36 | 0.45 | 0.11 | 0.19 |
| 1 | 0.45 | 0.39 | 1.08 | 0.67 | 0.11 | 0.41 |
| 2 | 1.41 | 0.95 | 2.01 | 1.04 | 0.13 | 0.52 |
| 5 | 4.7 | 4.5 | 5.9 | 5.0 | 0.22 | 0.61 |
| 10 | 10 | 10 | 11 | 11 | 0.32 | 7.4 |
| 50 | 56 | 31 | 64 | 68 | 31 | 3.2 |

The mean root length of *A. cepa L*, grown in each of the doped waters (CW or PW) were compared to the respective control length, and expressed as a percentage of the control values. These values for *A. cepa L.* are illustrated in Figs. 2-4 for both river water (CW and PW) as a function of the dissolved metal concentrations obtained by TXRF.

The relative change of the mean root growth showed very significant differences for both, the clean and polluted river water. The clean river water added with heavy metals inhibited the root growth at lower concentrations when compared to that caused by the polluted river water with the same metal addition. It might be due to the presence of more essential nutrients which could be due to the higher Biochemical Oxygen Demand (OBD) in the polluted water.

For copper enriched water (as $Cu(SO_4)$), the different response in root length to the same dosage in CW and PW, as shown in Fig. 2, was indicated that its toxicity was higher in clean water. It is well known that copper is an essential metal to both animals and plants. However, copper as $CuSO_4$ showed to be very toxic in the *Allium* test below the 0.03-mg.$L^{-1}$, showing a reduction of approximately 40% of the mean root length in

CW. Moreover, in the rapid decrease region of the root length, it was determined that the copper concentration in polluted water should be increased by a factor of 7.3 to obtain the same root growth inhibition effect corresponding to clean water. In addition, the excess of nutrients in polluted water induced a delay biological effect to high copper concentrations. Therefore, this effect could be used as an indicator for quality assessment in polluted river water.

For the Brazilian environmental legislation (CONAMA 20, 1986), the highest copper concentration recommended for river water class II is at 0.02-mg.$L^{-1}$. Moreover, the World Health Organization considers 1.5 mg.$L^{-1}$ as the highest copper permissible level in public water (WHO, 1971). These higher threshold values recommended for copper content in drinking or river water are in excess when compared with the low lethal concentration for the root growth in the *A. cepa L*.

Fig. 3 shows the root length response for zinc enriched water (as $ZnSO_4$). Zinc addition showed an opposite behavior compared to that of copper and lead. Zinc behaved like a nutrient for the *A. cepa L* up to 1.0 mg.$L^{-1}$ , and showed a toxic effect at higher concentrations with a reduction of 50% of the mean root length. For the Brazilian environmental legislation, the highest zinc concentration recommended for river waters class II is 0.18-mg.$L^{-1}$, below the lethal concentration for the *A. cepa L*.

Mean relative rooth length values as a function of dissolved lead concentrations are shown in Fig. 4. In clean water, a 50% reduction of root length occurred around 0.1 mg.$L^{-1}$, while for polluted water it was above 0.6 mg.$L^{-1}$. For lead, the lag in the reduction of root length in PW was very similar to that of copper. It was necessary to increase the lead concentration by a factor of 6 to get the same root growth inhibition effect corresponding CW.

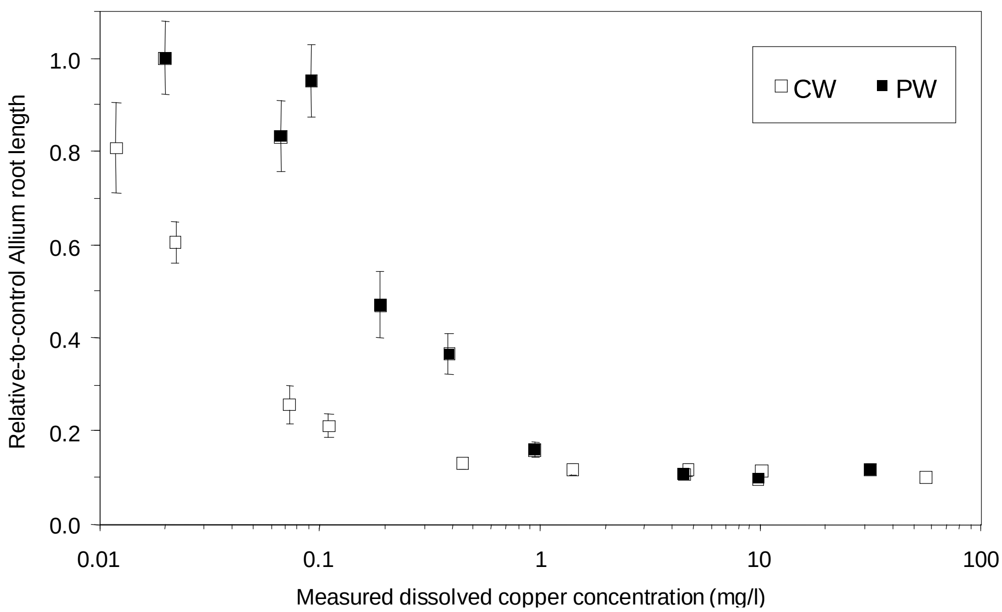


**Figure 2 -** Relative growth of *Allium* roots after the addition of $CuSO_4$ in various concentrations to the river water: CW - open squares and PW - filled squares.

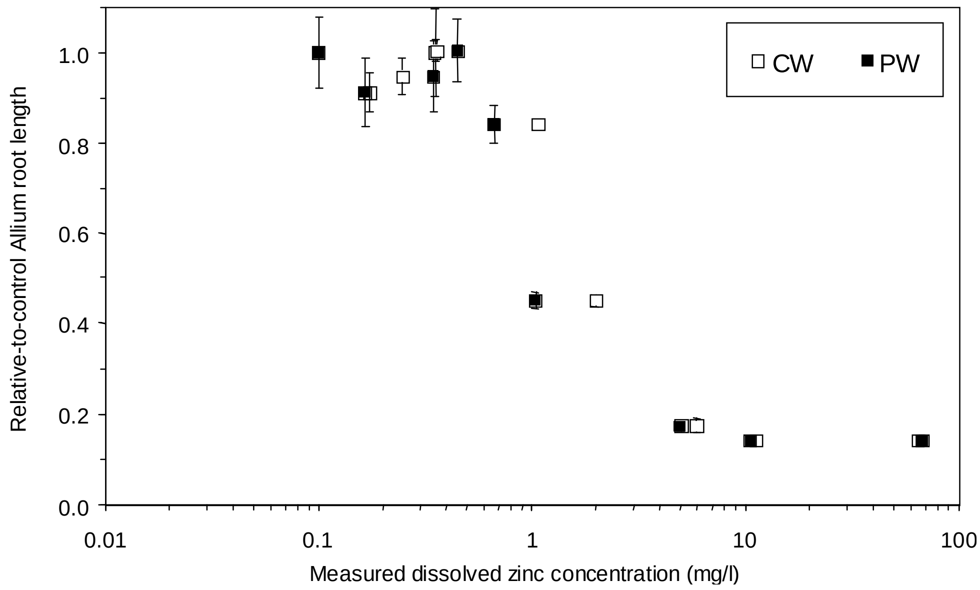


**Figure 3 -** Relative growth of Allium roots after the addition of ZnSO4 in various concentrations to the river water: CW - open squares and PW - filled squares.

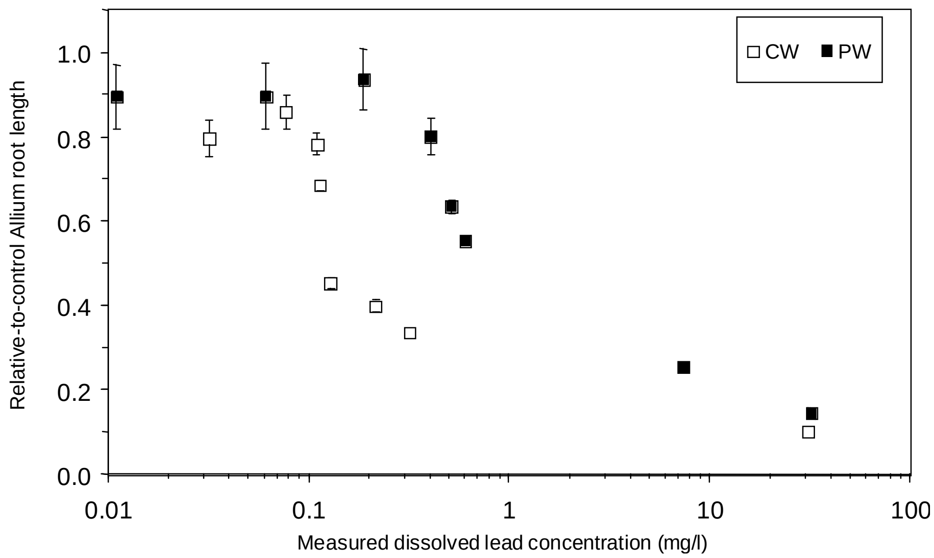


**Figure 4 -** Relative growth of *Allium* roots after the addition of $Pb(NO_3)_2$ in various concentrations to the river water: CW - open squares and PW - filled squares.

## CONCLUSION

This work reports the macroscopic effects on *A. cepa L* roots grown in clean and polluted river water added with metal compounds ($CuSO_4$, $ZnSO_4$ and $Pb(NO_3)_2$ ). TXRF analysis proved to be an effective way to determine the amounts of dissolved metals and permitted to verify the real amount of heavy metals in solution available for root growth. *A. cepa L* exhibited different sensitivities for each compound and each water (CW and PW). Copper and lead dissolved in clean river water showed root growth inhibition at 0.03 and 0.1 $mg.L^{-1}$, respectively (50% relative reduction of root length). On the other hand, zinc only showed a root length reduction effect at 1 $mg.L^{-1}$. In polluted water, growth inhibition effects showed a concentration lag factor for copper and lead probably due to the higher BOD. This effect could be used to estimate the eutrophization level of river water, based on the amount of increasing heavy metal concentration to get the same root length growth response in clean water. Due to the fast root growth and quick response to the tested chemicals, the *Allium* test could be useful for a practical water quality assessment.

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## RESUMO

O presente trabalho foi realizado utilizando a cebola comum (*Allium cepa L.*) como bioindicador da toxicidade de metais pesados em água de rio. As águas de teste foram coletadas em dois locais: na nascente e na foz do rio Toledo. Os bulbos de *A. cepa L.* foram cultivados em água de teste com nove níveis de concentração de cobre, zinco e chumbo de 0,1 a 50 ppm. Em laboratório a influência destes líquidos de teste em crescimento de raiz foi examinada durante cinco dias. Em todos os líquidos de teste o metal dissolvido contido foi medido pela técnica TXRF. Para líquidos de teste contendo 0,1-ppm de Cu dissolvido o crescimento da raiz foi reduzido em 50%. Entretanto, ocorreu a mesma redução para 1-ppm de Zn dissolvido. Para Pb dissolvido, o método do *Allium* teste revela toxidade acima de 0,1 e 0,5 ppm para a nascente e a foz do rio Toledo, respectivamente.

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