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Superoxide Dismutase Activity in the freshwater crustacean *Daphnia magna* exposed to Pb(II)

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Abstract

The aim of this work was the study of the toxic action of 2-hydroxyethyl-iminodiacetic Pb(II), [Pb – (HEIDA)] on the freshwater index-organism Daphnia magna. The results obtained from the toxicity experiments have been processed through the Trevors program, in the environment of BASIC, in order to assess the toxicity of the Pb(II) complex, expressed as EC50-48h. Based on the final results, it was found, that the Pb(II) complex had an EC50-48h value of 4.46 mg/L, leading to the conclusion that it is toxic enough to Daphnia magna.

In order to confirm that all experimental procedures were carried out properly, a reference experiment was conducted, using potassium dichromate as a toxic substance. The toxicity of potassium dichromate, was found to be within the acceptable limits, issued by ISO 6341, i.e. 0.85 mg/L (ppm).

Following determination of the EC50-48h of the Pb(II) complex, the study of its influence on the activity of the antioxidant enzyme Superoxide Dismutase of Copper - Zinc (Cu,Zn- SOD), isolated properly from the organism, was conducted. From the results of this study, it was found that the Pb(II) complex has practically no influence on the enzyme activity.

Keywords: Lead ecotoxicity, Daphnia magna, Cu,Zn-SOD enzyme activity, 2-hydroxyethyl-iminodiacetic Pb(II), microbiotests, EC_{50}-48h.

1. Introduction

Heavy metals have a worldwide distribution and they constitute a vital component of industrial discharges. Heavy metals are harmful at certain levels of exposure as a result of accumulation at relatively high concentrations in food chains of aquatic environments [1]. Fresh waters receive most of these toxic substances. Although aquatic ecosystems are equipped with a variety of physico-chemical and biological mechanisms to eliminate or reduce adverse effects of toxic substances, toxicants may evoke changes in development, growth, reproduction and behavior or may cause death in freshwater organisms [2].

Water fleas are among the most preferred animals for toxicity testing, especially so *Daphnia magna* (Cladocera), because of the large-body size [3]. *Daphnia sp.* is sensitive to poor water conditions and is most commonly used for monitoring water quality so that only safe water is released to the environment by industries. The species is widely distributed in North America, Europe, Asia and Africa. These cladocerans are important components of the zooplankton diet of fish and are mainly detritivores representing an important link in the food chain of virtually every inland water body converting phytoplankton/benthic plants, bacteria, fungi and decaying organic matter into animal tissue that can be used by larger animals (fish) [4].

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Sandholm et al. [5] reported that residue levels in fish more than doubled when the fish are exposed to selenium in both food zooplankton and water as compared to water alone.

Aquatic organisms are currently being exposed to multiple chemical contaminants with different mechanisms of toxicity, each contributing to a final overall adverse effect. Consequently, in ecological quality monitoring programs, the integration of chemical data with biological responses (biomarkers) is strongly recommended to characterize effects of contaminants to organisms. At the biochemical level, biomarkers were included in studies on enhanced production of reactive oxygen species (ROS) as a general pathway of toxicity induced by many redox cycling chemicals (hydrocarbon quinones, nitro-aromatics, biphenyls [6] and many other compounds leading to a condition of oxidative stress. These ROS include superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and the highly reactive hydroxyl radical ($OH^{\cdot}$).

To minimize oxidative damage to cellular components, organisms have developed antioxidant defense mechanisms. One of the most important antioxidant enzymes is superoxide dismutase (SOD, EC 1.15.1.1), which converts the superoxide anion radical ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2).

The development of biomarkers of oxidative stress as a polluted-mediated mechanism of toxicity requires knowledge of how antioxidant biochemical systems and target molecules are influenced by model pollutants. The aim of this study is to use the Cladoceran crustacean *D. magna* as a model organism to study the biochemical responses of oxidative stress through the enzyme (Cu,Zn- SOD) of the organism during its exposure to the compound 2-hydroxyethyl-iminodiacetic Pb(II), [Pb – (HEIDA)]. In this work, a specific compound of Pb(II) was used, as a toxicant, which is synthesized and well characterized physicochemically in our lab, in order to better assess the toxicity of the metal vs. *D. magna*, avoiding the influences of impurities, in case of using a Pb(II) compound from the marketplace.

2. Materials and methods

The compound 2-hydroxyethyl-iminodiacetic Pb(II), [Pb – (HEIDA)], was synthesized in our laboratory. All other chemicals were of analytical grade and were obtained from Aldrich. The index-organism *Daphnia magna*, comes to the market in the form of Toxkit (DAPHTOXKIT FTM MAGNA), which contains all the essential materials, for the implementation of toxicity experiments for the various chemical substances (microbiotests). Standard freshwater (SFW)(ISO 6341) or distilled water was used throughout the experimental runs.

Figure 1: The toxicity index-organism *Daphnia magna*

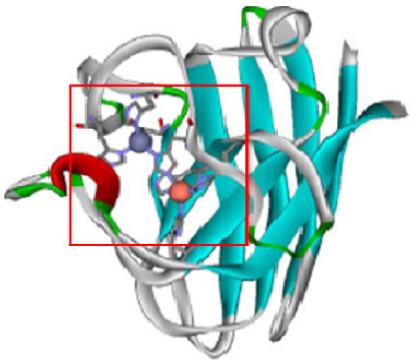

Figure 2: The structure of the Cu,Zn-SOD enzyme

The toxicity of the Pb(II) compound on *D. magna* juveniles was determined, following standard operating procedures according to ISO 6341. The EC_{50}-48h was estimated to be 4.46 ppm within the 95% confidence interval. Finally, the total SOD enzyme activity of *D. magna*, exposed to various concentrations of the Pb(II) compound, was measured and assessed. A number of *Daphnia* juveniles (70), were homogenized at 4 ^{o}C in a 1:4 wet wt./buffer volume ratio in 100 mM phosphate buffer, pH 7.4, containing 100 mM KCl and 1 mM EDTA [7].

Homogenates were centrifuged at 10,000g for 10 min and the supernatants were immediately used as enzyme source. Oxygen peroxide radicals were generated following the method of Sichel et al [**8**]. Total SOD activity was determined by the method of Beauchamp and Fridovitz (1971), based on the nitro-blue tetrazolium (NBT) reduction by the superoxide anion $O_2^{\cdot-}$ to give blue formazan [9].

This reaction was followed spectrophotometrically, with the blue formazan revealing its characteristic UV-Vis peak at λ_{max} = 560 nm. This method had been used earlier for the evaluation of the SOD-like activity of Cu(III)-polypeptide complexes, serving as SOD models [10].

The specific Pb(II) compound was proven to be toxic enough for *D. magna*, in relation to other Pb(II) compounds [1, 11]. The influence of the Pb(II) compound on the Cu,Zn-SOD of the organism enzymic activity was negligible, i.e. neither enhancement nor reduction of the enzymatic activity was observed.

4. Conclusion

The index-organism *Daphnia magna* offers a convenient and also reliable tool, not just for the determination of the toxicity of Pb(II)(and other metals as well) to model invertebrate-organisms, but also for the assessment of the influence by the metal(s) on the organism SOD enzymatic toxicity. In this case, the SOD activity of *Daphnia magna* juveniles was found to remain practically stable. Further experiments with various metals are necessary, in order to elucidate the mechanisms, underlying the toxicity mechanisms in *Daphnia magna*.

In any case, the results suggest that an accurate and realistic picture on the molecular toxicity of Pb(II)(and other metals) could be deduced, only when well-defined species are employed, like our Pb(II) compound, in the toxicity bioassays.

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