XIX CONGRESO de la SEBC
BOADILLA DEL MONTE (Madrid)
26, 27, 28 y 29 de octubre de 2021

A comparative evaluation of bioaccumulation and induction of oxidative stress by waterborne copper (Cu) and arsenic (As), as dissolved forms (DF) and nanoparticles (NP), in larvae of European seabass (*Dicentrarchus labrax*, L.)

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INTRODUCTION

In the recent years, nanotechnology has produced a large number of nanomaterials and metal nanoparticles (NPs) with new useful properties, leading to their broad spreading throughout industrial and household products. As a result, NP have increasingly entered into natural aquatic environments so that a scientific concern arises about the safety of NPs for human health and the environment, as they are considered hazardous emerging pollutants. While toxicity of metals in their dissolved forms (DF) have been addressed to a great extent, information on that related to the nanoparticles (NP) forms is largely. **Our aim** was to assess the potential induction of oxidative stress and the antioxidant enzymatic response induced in *Dicentrarchus labrax* larvae, one of the most important commercial fish widely cultured in the Mediterranean, by copper and arsenic under these different chemical forms.

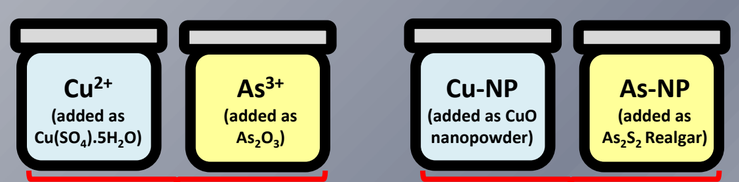
METHODOLOGY

Experimental design

20 Tanks (1-10, 24h; 11-20: 96h) for each metal (Cu or As) and for each of their chemical forms (Dissolved forms or Nanoparticles) (5 concentrations x 2 replicates)

50 Larvae in each tank

- Tank 1,6,11,16: 0 ppm
- Tank 2,7,12,17: 0.01 ppm
- Tank 3,8,13,18: 0.1 ppm
- Tank 4,9,14,19: 1 ppm
- Tank 5,10,15,20: 10 ppm



Tanks for Bioassays

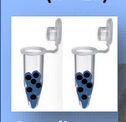

Fish samples


Water samples

Chemical Determinations

Cu and As analysis in fish samples

Larvae pool (n = 20) → Freeze - dried → Digested by microwave heating with HNO_3 (sup) 65% and H_2O_2 (sup) 30%


2 replicates


40 larvae


ICP-MS (Inductively Coupled Plasma Mass Spectrometry)

Biochemical Analysis

At the end of 24 or 96 h of exposure, the larvae were collected from each tank, quickly frozen in liquid nitrogen and stored at -80ºC until their use in biochemical tests. 10 larvae were used for the biochemical analyzes (in two pools of 5) and 40 for the metal analyzes (in two pools of 20). Biochemical analyses were carried out on a subcellular fraction. For this, the larvae were chopped and homogenized in cold 0.1 M potassium phosphate buffer (pH 7.4) with EDTA 0.05 mM using a glass tissue homogenizer. The supernatant constituted the sample for biochemical studies. Samples were centrifuged for 30 min at 12,000 g at 4 °C.

Tissues → Homogenization: 0.1 M Potassium Phosphate Buffer (pH 7.4) + 0.05 mM EDTA → Centrifugation (for 30 min at 12,000 g a 4ºC) → Supernatant

- Levels of hydroperoxides: Lipid peroxidation was estimated according to the xylenol orange assay as described by Gay and Bebicki (2000).
- Superoxide dismutase Activity (SOD): SOD activity was determined based on the ability of the enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) (Crouch et al., 1981), which was generated by xanthine oxidase.
- Catalase Activity (CAT): CAT activity was determined by the method of Aebi (1983), by measuring the decrease of H_2O_2 concentration.
- Protein concentrations were determined according to the method of Bradford, (1976) using BSA as a standard.

Statistical Analysis The data are expressed as means ± SE (standard error). Significant differences were determined by one way ANOVA followed by the Duncan post-hoc test. Significant differences were considered at $p< 0.05$.

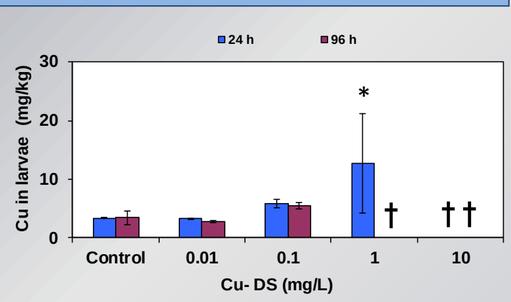
RESULTS AND DISCUSSION

METAL CONCENTRATIONS IN LARVAE

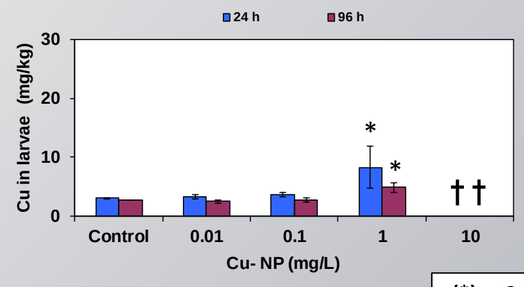
Mortality. Total Mortality occurred in larvae exposed to Cu-DF 1 mg/L at 96h and 10 mg/L at both 24 and 96 h to Cu-DF and Cu-NP. No mortality was observed in the other treatments.
Cu appears to be significantly accumulated in larvae tissues since the 1 mg/L but in a lesser degree for Cu-NP.
As significantly accumulated at 10 mg/L with a high concentration after the 96 h treatments. respectively.
In both cases, the NP exposure resulted in a less accumulation in larvae tissue.

EXPOSITION TO COPPER:

DISSOLVED Cu

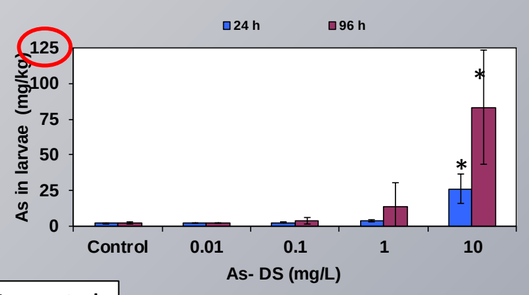


Cu NANOPARTICLES

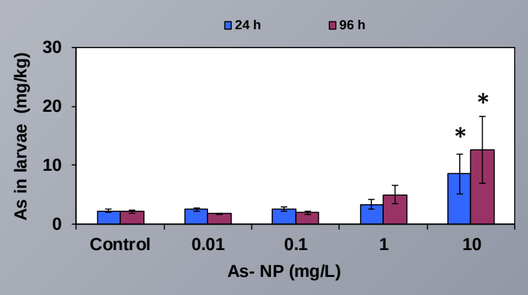


EXPOSITION TO ARSENIC:

DISSOLVED As



As NANOPARTICLES



(*) $p<0.05$ *vs* control

BIOMARKERS OF OXIDATIVE STRESS

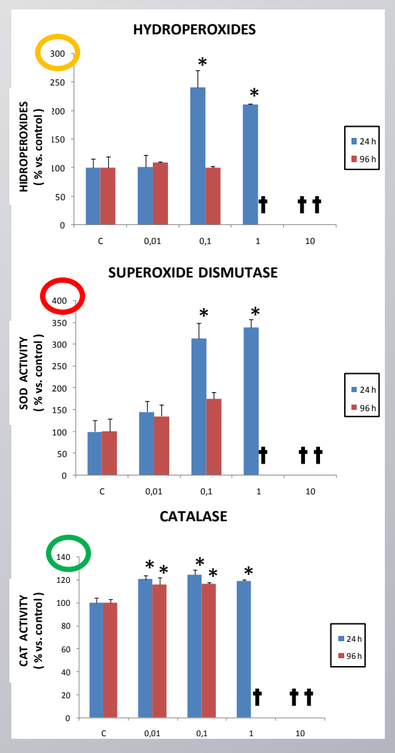
Lipid peroxidation (HP content):
Both Cu-DF and Cu-NP increased hydroperoxides from the 0.1 mg/L at 24h. However, no effects were observed at 96 h by both chemicals forms.

Superoxide Dismutase (SOD) activity paralleled concomitant increases in Hydroperoxides levels.

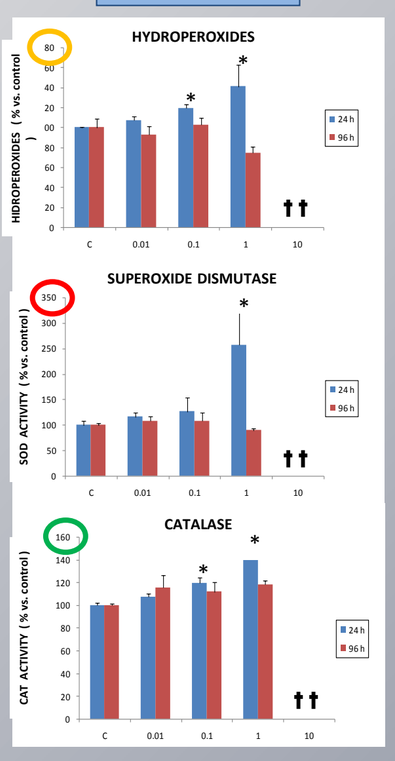
Catalase (CAT) activity was increased since 0.01 mg/L and 0.1 mg/L for Cu-DF and Cu-NP at 24 h, respectively. Cu-DF also increased CAT activity at 96 h from 0.01 mg/L. However, no effects were observed at this period by Cu-NP.

Response of *D. labrax* larvae exposed to COPPER:

DISSOLVED Cu

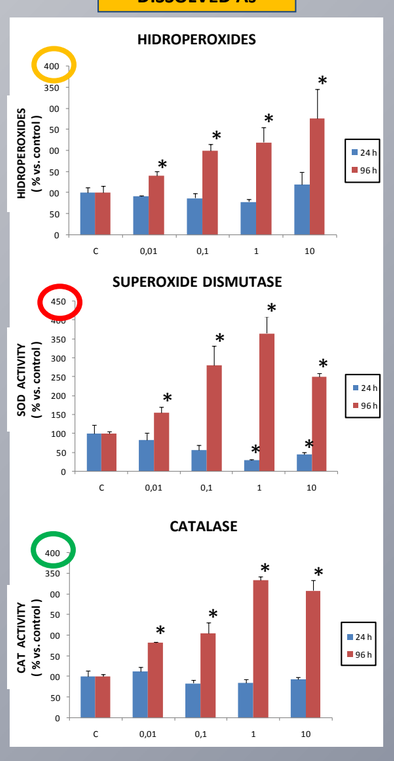


Cu NANOPARTICLES

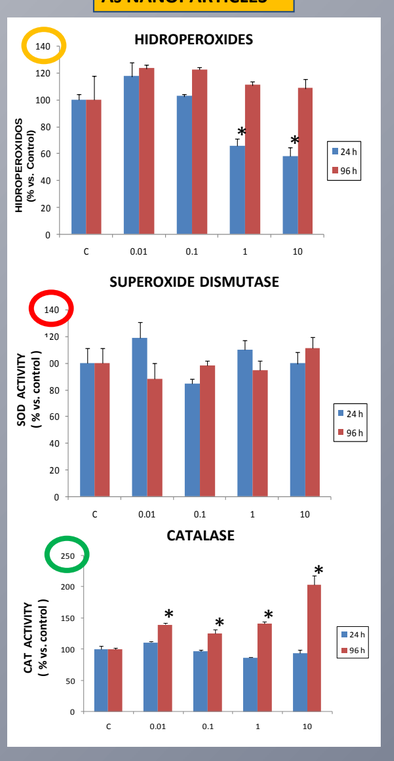


Response of *D. labrax* larvae exposed to ARSENIC:

DISSOLVED As



As NANOPARTICLES



Lipid peroxidation (HP content):
No oxidative damage was detected for any form of As after 24h; it was induced by As-DF since the lowest concentration after 96h, while no effects were observed by As-NP.

Superoxide Dismutase (SOD) activity decreased in As-DF after 24h, but increased after 96h; As-NP did not alter SOD activity.

Catalase (CAT) activity was stimulated at 96h by As-DF and As-NP.

CONCLUSSIONS

These results suggest that a differential induction of oxidative damage and of the enzymatic antioxidant response was observed for the two elements and their respective chemical forms. Importantly, as previously stated for other metals, the response elicited by the dissolved forms of Cu and As tended to be more potent than the NP forms.

This work has been supported by the Project RNM-6641 RNM-6641 from the "Consejería de Economía, Innovación y Ciencia de la Junta de Andalucía" and the Project CTM2010-17474 from the "Ministerio de Ciencia e Innovación"