

# Investigating bioaccumulation of heavy metals and speciation of copper in oysters (*Crassostrea gigas*) using XANES and EXAFS spectroscopies

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Copper (Cu) concentration in oyster tissues can be particularly high due to contamination, leading to extreme green/blue colors. This raises key questions regarding the detoxification of metals in oyster tissues and their chemical speciation in these tissues. This study investigated heavy metal bioaccumulation and copper speciation in selected oyster tissues using XANES/EXAFS spectroscopic methods. Moreover, heavy metal distribution and concentration profiles from several oyster tissues were also examined using ICP-MS, FTIR, and EDX spectroscopy. The XANES and EXAFS results revealed that copper existed as copper(II)oxide (CuO) in contaminated oysters formed Cu clusters with Cu–Cu and Cu–O interatomic distances of 2.93 and 1.97 Å, (coordination numbers of 20.21 and 1.00), respectively. In normal oysters, the less abundant copper sulfide (CuS) formed clusters with a Cu–S (thiolate) bond distance of 2.089 Å and a coordination number of 4.56. The concentrations of Cu and Zn were extremely high in oyster tissue samples ranged within 1100–1400 and 500–700 mg/kg, respectively. This study provides direct information on heavy metal speciation in contaminated oysters, demonstrating that oysters might detoxify bio-accumulated Cu in heavily contaminated environments, by storing it in oxygen-bonded compounds.

**Keywords:** *Oysters (Crassostrea gigas), Heavy Metals, Bioaccumulation, XANES/EXAFS*

## Introduction

Oysters are highly nutritious and are therefore an important dietary constituent, derived from the marine ecosystem. However, oysters accumulate extremely high amounts of heavy metals compared to other aquatic invertebrates. Green/blue coloured oysters are mostly located in the coastal and estuarine regions with significant contamination with heavy metals like copper (Cu), zinc (Zn), and cadmium (Cd) among others. Oysters harvested from contaminated areas are a potential risk to public health when consumed by humans. The intake of oysters contaminated with heavy metals may cause cancer, damage to the kidneys, the immune system, and the central nervous system [1]. The X-ray absorption spectroscopy (XAS) methods such as X-ray-absorption near edge structure (XANES) and the extended X-ray absorption fine structure (EXAFS) spectroscopic can provide information about in-situ and direct metals speciation. High concentration of metals in the samples is required to obtain high-standard XAS data. In most studies, oysters have been found to accumulate high Cu concentrations, which enable application of XAS methods for dictating the Cu species [2].

In the present study, the XANES and EXAFS techniques were used to provide insights into the speciation of Cu in contaminated-oyster tissues.

## Experiments

Oyster samples were brought in the laboratory and depurated in filtered seawater for 1 day. Stainless steel blades were used to excise oyster tissues which were then washed using Milli-Q water to remove impurities. The tissues were dried in an oven at  $105 \pm 2$  °C for 12 h to a

constant weight. A composite sample made from oyster tissues were crushed into tiny tablets using a mechanical punch, before being collected for XANES/EXAFS experiments.

## Results

The Cu *K*-edge XANES spectra of various Cu standards was investigated to determine the most likely forms of Cu present in the contaminated oyster tissues. Assuming that all examined standards were prepared using Cu(II), the Cu *K*-edge XANES spectra of all standards demonstrated a similar absorption edge ranging from 8780 to 9620 eV as shown in **Figs. 1(a) and 1(b)**. Generally, the Cu species were bound to O/S and N-containing ligands. However, Cu binding to the ligand resulted in slight shifts of the peaks representing Cu coordinated with S and Cu bound to O ligands, at 8979 eV. These results are consistent with the notion of a strong covalency between Cu–S and Cu–O bonding. Additionally, the Cu(II)O spectra displayed characteristic peaks at approximately 8977 to 8978 eV. The presence of O ligands for Cu binding is consistent with the results obtained by Tan et al. (2015), who reported that Cu formed a coordination compound with O and S ligands [2]. The photon energy positions of Cu in the Cu-contaminated oyster tissues samples overlapped with that of CuS and CuO. This observation indicated that the valency of Cu in CuS is Cu(II) instead of Cu(0). In general, the absorption edge position of Cu oxidants is higher than that of metallic Cu. This in turn suggests that the shift in absorption edge position of Cu increases with the increase in valency of Cu. Thus, it can be concluded that the 1.5 eV absorption edge position shift is caused by

an increase in each Cu oxidation number. Using this principle, it is convenient to calculate the valency of Cu in the oyster tissues by a comparison with several Cu standards. An absorption peak was found in the post edge of Cu *K*-edge absorption for the oyster tissues. This can be attributed to electron migration from ground state to the excited state (e.g.  $1s \rightarrow 4d$ ). The aforementioned occurrence is sensitive to the first shell symmetry of the neighboring atoms surrounding a Cu central atom. Exposure to photon energy in the post edge of Cu *K*-edge allows the electrons to migrate to a higher energy potential (i.e.  $1s \rightarrow 4p_{xy}$ ). The bond distances of Cu–O and Cu–S in Cu-contaminated oysters were also fitted in EXAFS spectra (Figs. 1(c) and 1(d)). The first shells of the oyster tissues samples were found to fit well with the molecular structure of CuS and CuO. The EXAFS spectra of Cu in the oyster tissues showed that the Cu(II)oxide in the oyster tissues formed Cu aggregates with Cu–Cu and Cu–O interatomic distances of 2.93 and 1.97 Å, respectively. The coordination number for Cu–Cu and Cu–O in the first shell was 20.21 and 1.00. Additionally, CuS in the oyster tissues formed clusters with a Cu–S interatomic distance of 2.089 Å and a coordination number of 4.56 in the first shell. With reference to the interatomic distance, speciation of Cu in the oyster tissue samples might be distinctly separated into two species of Cu–S/O bonds.

## Discussion

Specifically, in normal oysters and at lower concentrations of Cu, it existed as Cu(I) and thiolates (S bonded species) however, heavy metal-contaminated oysters, Cu existed primarily as Cu(II) and Cu–O bonded types. These results are conforming to the Lewis acid/base categorization where Cu(I) is defined as a class B metal with high attraction for S donors, while Cu(II) is known to be a borderline metal with higher attraction for O donors. Considering that Cu is an oxidative stressor for marine creatures, it can be speculated that the reactive oxygen species produced by high Cu exposure surpassed the ability of the oysters to manage it. Consequently, the extremely bio-accumulated Cu might not be effectively decreased or sustained as Cu(I) and was kept by binding to the O donors as Cu(II).

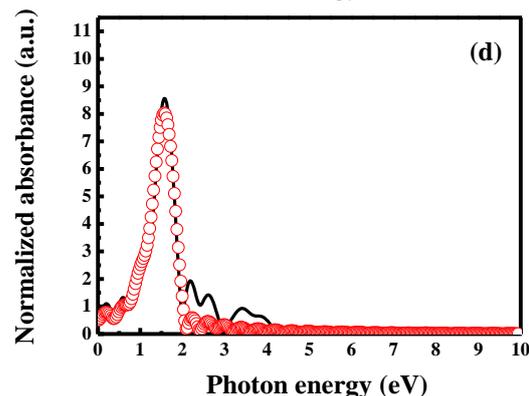
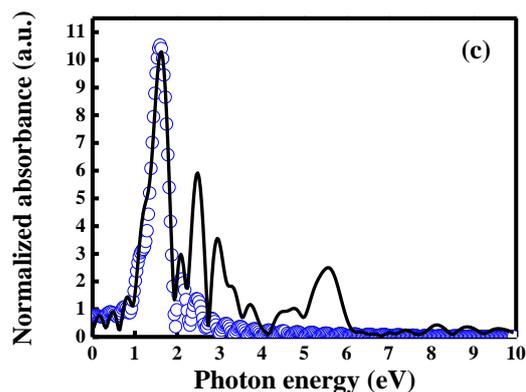
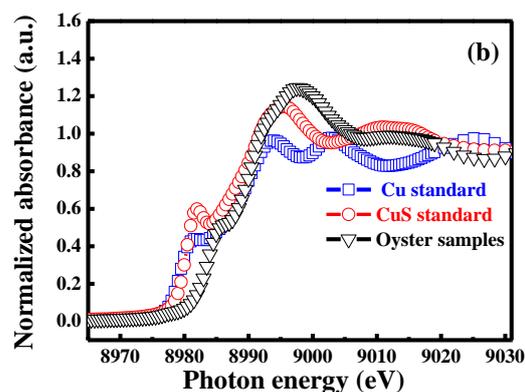
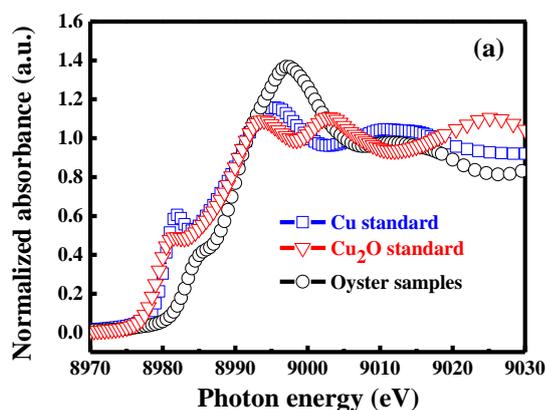


Fig. 1 (a)–(b) XANES spectra of Cu-contaminated oyster tissue samples compared with that of Cu standards, (b)–(c) EXAFS Cu *K*-edge Fourier transformed spectra of Cu-contaminated oyster tissue samples of contaminated oyster tissue samples.

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