Facile synthesis of Cu/Ls MOF for laccase-like oxidative decomposition of phenols and identification of toxicity effects for C. elegans

Hui Li
Jilin University
Feng Guo
Jilin University
Zhendong Fu
Jilin University
Liping Wang (wanglp@jlu.edu.cn)
Jilin University https://orcid.org/0000-0002-3458-4513

Research Article

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Abstract

Certain compounds in chemical products adsorb heavy metals co-occurring in surface waters and thereby modulate their toxicity for invertebrates. Sodium N-lauroylsarcosinate salt (Ls) is widely used as a medium additive in detergents and can chelated with certain metal ions. In this study, we constructed metal-organic framework by the co-assembly of Ls and Cu (Cu/Ls MOF), which has laccase like activity. The morphology of Cu/Ls appeared spherical or ellipsoidal with a diameter in the range of 50–500 nm. Cu/Ls MOF followed the typical Michaelis-Menten model and showed laccase-like activity and oxidizes phenol-containing substrates, such as phenol, 4-iodophenol, and 2,4,5-trichlorophenol. To understand the toxicity of Cu/Ls exposure, the *Caenorhabditis elegans* (*C. elegans*) was exposed to various concentrations of Cu/Ls, and the impact on physiological level was determined. High dose of Cu/Ls increased the levels of reactive oxygen species (ROS), decreased the exercise activity of the *C. elegans* and inhibited the growth of their larvae. Cu/Ls had strong binding interaction and the ability of Cu/Ls to oxidize pollutants may be a form of self-regulation by nature. The biological toxicity of Cu/Ls is low under high concentration conditions, and the dose of MOF that may be formed by the discharge of daily washing is less harmful to the environment.

1. Introduction

Phenols are widely utilized in both the industrial and academic research environment (Wu et al., 2016). with the large demand for chemical, a large amount of wastewater containing phenol is discharged into the aquatic environment, and which lead to phenol pollution of the rivers and ocean (Li et al., 2019a). Phenol as a common chemical ultimately constitutes a serious environmental hazard (Li et al., 2019b). Currently, the removal of high concentrations of phenol from wastewater consist mainly of adsorption and solvent extraction (Marrot et al., 2006). Creating catalysts that mimic enzymes to replace natural enzymes for practical applications has become a hot research topic.

As a common amide-containing surfactant, sodium N-lauroylsarcosinate (Ls) can be found in detergents, especially in shampoo, bubble-bath pastes, and cleaning cream (Ghosh and Dey, 2015). In addition, Ls is widely used in the construction industry as an excellent corrosion inhibitor (Perinelli et al., 2020). Ls interacts with other ions, such as heavy metals, and is likely to concentrate such ions and to potentially modulate their toxicity. Surfactants possess the ability to self-assemble structures and have potential applications in drug delivery and cosmetics. (Li et al., 2014). Aqueous mixtures of Ls and the cationic surfactant N-dodecylpyridinium chloride can produce small stable vesicles (Ghosh and Dey, 2011), which can be used as an effective drug delivery vehicle. Starting from these studies, when investigating laccase mimics, we observed that Ls formed complexes with Cu ions and then could effectively catalyze the reaction of phenol derivatives with oxygen. Since laccases can catalyze the oxidation of various compounds containing phenolic structures (Su et al., 2018), enzyme-mimicking catalysts have received attention as possible replacements of natural enzymes in the field of environmental science (Liang et al., 2017; Shams et al., 2019). In amide-containing surfactants, the amide group is an easily accessible source of electrostatic, H-bonding and hydrophobic interaction forces (Perinelli et al., 2020), that provides

the basis for the construction of different complexes. At the same these interactive forces are responsible for the strong stability of compounds from Ls and metal ions in the form of a metal-organic framework (MOF). Based on our observations, one can assume that the Cu/Ls active in the decomposition of phenol are forming laccase mimics.

To understand the toxicity of Cu/Ls, the nematode *Caenorhabditis elegans* (*C. elegans*) was exposed to various concentrations of this material. In the natural environment, *C. elegans* inhabits the surface layer of soil and feeds on bacteria, which makes it a powerful model for environmental toxicology (Nagar et al., 2020).

This paper the nature of the enzymatic reaction properties of Cu/Ls and its structure. Finally, its possible toxicity in the environment was assessed by using the *in vivo* model *C. elegans*.

2. Experimental

2.1 Chemicals

Copper(II) sulfate ($CuSO_4 \cdot 5H_2O$, 99.9%), 4-aminoantipyrine (4-AP, 98%), 2,4-dichlorophenol (2,4-DP, 98%), and 2-(N-morpholino)ethanesulfonic acid (MES, 99.5%) monohydrate were obtained from Aladdin Biochemical Technology (Shanghai, China). sodium N-lauroylsarcosinate (98%) was obtained from Beijing Dingguo Changsheng Biotechnology (Beijing, China).

2.1 Synthesis of Cu/Ls MOF

Briefly, MOFs are formed by mixing (25 mM, 500 μL) and $CuSO_4$ or other metals (50 mM, 500 μL) in solution to form precipitate. Subsequently, the reaction precipitate was collected by centrifugation (12000 $r \cdot min^{-1}$, 5 min) and washed with ultrapure water to purify precipitate. Cu/Ls MOF was obtained by drying the precipitate at 60°C. Before the subsequent experiment, the aqueous solution of Cu/Ls MOF would be sonicated for 5 minutes to obtain a fine dispersion.

2.2 Characterization of Cu/Ls MOF

The concentration of Cu/Ls MOF used for structural characterization and enzymatic properties was 100 μg-mL-1 in water. Scanning electron microscopy (SEM) was used to examine the morphology and dimensions of MOF, which was performed on a Hitachi S-4700 microscope (Hitachi, Ltd, Japan). FTIR spectroscopy X-ray photoelectron spectroscopy was tested using Nicolet-6700 IR spectrometer (Nicolet, USA) and XPS spectrometer model ESCALAB250XI (Thermo Scientific, USA), respectively. XPS Peak Fit Version 4.0 software was used for data analysis and processing.

2.3 Catalytic activity assay

Laccase catalytic activity of the Cu/Ls MOF was measured spectrophotometrically at 510 nm by following the oxidation of 2,4-DP by using as the substrate and 4-AP as the chromogenic reagent (Guan et al., 2020; Liang et al., 2017). Briefly, 2,4-DP (100 μL, 1 $mg \cdot mL^{-1}$) and 4-AP (100 μL, 1 $mg \cdot mL^{-1}$) were

mixed with MES buffer (700 μL, 30 mmol·L^{-1}, pH 7). Then, Cu/Ls MOF (100 μL, 1 mg·mL^{-1}) was added to the mixture. The reaction was maintained at 25°C and monitored at 510 nm after 1 h (UV-2501PC, Shimadzu, Japan). The amount of change in chromogenic produced per unit time is defined as the reaction rate (*V*, in mmol·L^{-1}·min^{-1}).

2.4 Analysis of stability

All these experiments refer to previously reported (Guan et al., 2020; Liang et al., 2017). Briefly, In the pH stability experiments, MOF was separately suspended in advance in a range of pH (1–12) and the absorbance was measured after 6 hours. In the temperature stability experiments, Cu/Ls dispersions were incubated at different temperatures (30–90°C) for 30 min after measuring. The effect of ionic strength on catalytic activity was measured by mixing Cu/Ls with different concentrations of NaCl. The long-term storage stability was determined by measuring the activity of dispersed Cu/Ls after 100 days of storage at room temperature. For these experiments, the pretreated Cu/Ls were added to the reaction system and then maintained at 25°C for the reaction and measured at 510 nm after 1 h.

2.5 Determination of Enzyme Kinetic Parameters

The initial reaction rates can be calculated for different concentrations of 2,4-DP (10, 20, 40, 60, 80 and 100 μg·mL^{-1}) mixed with Cu/Ls for the reaction. The kinetic parameters *(Km* and *Vmax*) were calculated by the Michaelis-Menten. 13.6 L·$mmol^{-1}$·cm^{-1} (ε) was used in the *Km* calculations.

2.6 *C. elegans* strain and culture

N2 *C. elegans* were purchased from the Caenorhabditis Genetics Center (CGC, University of Minnesota, USA) and maintained at 20°C. *C. elegans* were grown in Petri dishes on nematode growth medium (NGM) and fed with *E. coli* OP50.

2.7 Acute toxicity of Cu/Ls exposure in *C. elegans*

Synchronized L4 worms were exposed to various concentrations of Cu/Ls for 24, 48, and 72 h in K-medium (0.31 g NaCl and 0.24 g KCl in 100 mL deionized water) (Munro et al., 2020). 20 worms were added to each well of a 24-microtiter plate. Following exposure, the live and dead nematodes were scored. The experiment was repeated three times independently.

2.8 Measurement of reactive oxygen species

Endogenous reactive oxygen species (ROS) levels were measured using 2',7'-dichlorofluorescein diacetate (H_2DCF-DA) (Yang et al., 2018). Worms were treated with or without 100 μg·mL^{-1} Cu/Ls for 24 h and then collected. Then, the nematodes were incubated with 100 μM H_2DCF-DA for 30 min. Fluorescence intensity was measured at excitation and emission wavelengths of 470 and 550 nm, respectively. The assay was performed in three independent trials. The software program ImageJ 15.2v. was used to quantify the fluorescence intensity (Schneider et al., 2012).

2.9 Analysis of body length

L1 stage *C. elegans* pretreated with or without Cu/Ls MOF for 24, 48 and 72h. The worms were recorded on a stereoscope model BK1201 (Chongqing COIC Industrial Co., Ltd., China). The body length of the worms was measured using Image J 15.2v software (Schneider et al., 2012).

2.10 Body bend assay

To measure body bending, 30 worms treated with or without 100 μg·mL^{-1} Cu/Ls for 24 h were placed on M9 buffer without food, and the number of sinusoidal curves made during locomotion in 20 s was scored. This assay was repeated independently three times.

2.11 Reproduction assay

Synchronized L4 larvae (n = 3) were randomly transferred to fresh NGM plates and pretreated with or without 100 μg·mL^{-1} Cu/Ls MOF. They were transferred onto a fresh NGM plate every 24 h. The eggs were then allowed to hatch and were counted at the L2 or L3 stage. The total number of progenies was referred to as the initial reproduction.

2.12 Pharyngeal pumping assay

For pharyngeal pumping experiments (Li et al., 2021), worms (n = 10) were treated with or without 100 μg mL^{-1} Cu/Ls for 24h on NGM plates. The pharyngeal pumping was recorded on the COIC stereoscope mentioned earlier and was counted for 10 s.

2.13 Statistical analysis

Statistical analysis was performed using Prism 7 software from GraphPad. Data was analyzed by Student's t-test, and values were presented as mean ± SD. Statistical differences were considered significant at $p < 0.05$ (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, comparison to 0 μg·mL^{-1}, ns: no significance).

3. Results And Discussion

3.1 Cu/Ls MOF with Laccase-like Activity.

The UV–vis spectrum showed that each compound alone does not absorb light in the visible region (Fig. 1A). The red product can be detected by absorbance at 510 nm. As shown in Fig. 1A, the reaction system changed from colorless to red after the addition of Cu/Ls MOF, indicating that the material has a catalytic ability similar to laccase. Because residual free Cu^{2+} in Cu/Ls MOF may cause false positive results, another experiment was designed to demonstrate that Cu/Ls MOF possesses laccase-like activity. The dispersed Cu/Ls were centrifuged, and the supernatant and precipitate were removed separately to detect catalytic activity. The supernatant exhibited almost no activity in catalytic test. The precipitate was still active, which indicated that the catalytic activity belonged to Cu/Ls MOF rather than the free Cu^{2+} (Fig. 1B). Kinetic measurements were conducted with natural laccase and Cu/Ls MOF at different substrate concentrations to obtain the enzyme kinetic parameters. The *Km* and *Vmax* values were listed

in Table 1 and suggested Cu/Ls MOF had similar activity and enzymatic properties compared to natural enzymes.

The stability aspect of Cu/Ls MOF was systematically investigated under various harsh conditions. Unexpectedly, the activity of Cu/Ls MOF increased by 8.5-fold at a NaCl concentration of 500 mM compared control group (Fig. 1C). The high concentration of NaCl 500 mM NaCl may cause a decrease solubility of 2,4-DP and 4-AP via the salting-out effect(Liang et al., 2017). Subsequently, these compounds might have been preferentially adsorbed by the Cu/Ls MOF and then converted. Next, the Cu/Ls MOF was exposed to MRS buffers of different pH (ranging from 1–12) for 6 h (Fig. 1D). In these experiments, the activity of Cu/Ls MOF was highest at pH 7. In the following investigations on the effect of temperature, the Cu/Ls MOF was exposed to 30 to 90°C for 30 min before activity testing was performed. As shown in Fig. 1E, the activity of Cu/Ls MOF was influenced only to a limited extent by the thermal treatment. Compared to the reaction after exposure to 30 ℃, the activity decreased only by 36% after treatment at 90 ℃. Concerning the long-term stability of the catalyst, Cu/Ls maintained a favorable catalytic activity with a retention of 95% after 100 days in a pure water solution (Fig. 1F).

Table 1
The enzyme kinetic parameters of laccase and Cu/Ls.

Catalysts	*Km*(mM)	*Vmax*(mM min^{-1})
Cu/Ls	3.4 ± 0.89	0.0011 ± 0.00015
Laccase	0.15 ± 0.021	0.012 ± 0.00053

3.2 Effect of metal ions and substrates on the MOF Activity

Since sewage contains different metal ions (Gogoi et al., 2020), we investigated whether the combination of other metal ions with Ls exhibited a laccase-like activity. Although precipitation with Ls ligand was observed with all tested metal ions (Fig. 2A, top tubes), their activity was very low except for Cu^{2+} (Fig. 2A, below tubes). Accordingly, the activity of Cu^{2+} was significant.

To test the Cu/Ls MOF activity toward different substrates, 4-AP was mixed with selected phenolic compounds (Fig. 2B), of which the chemical structures are displayed in Fig. 2C. The results of these experiments demonstrate that Cu/Ls MOF possesses the capability to oxidize each of the phenolic compounds.

3.3 Structural characterization of Cu/Ls MOF

The structural morphology of Cu/Ls MOF was studied by using SEM. The morphology of Cu/Ls MOF exhibited smooth surface, spherical and ellipsoidal micelle-like structures of different sizes with approximately 50–500 nm (Fig. 3A and 3B).

Figure 2 showed the FT-IR spectra of Ls and Cu/Ls MOF. The spectrum of the Ls featured a characteristic peak at 1616 cm^{-1}, due to the asymmetric stretching vibration of the carboxylate moiety, along with symmetric stretching vibrations at 1402 cm^{-1} (Li et al., 2018). The splitting of the broad C = O asymmetric stretching vibration band at peaks 1616 cm^{-1} reflected the formation of Cu-O coordination (1598 and 1660 cm^{-1}) (Liang et al., 2017; Shams et al., 2019). The FTIR spectrum of Cu/Ls MOF in the wavelength range of 4000 – 500 cm^{-1} confirmed that the Cu/Ls MOF was formed through the coordination of Ls with Cu ions (Fig. 3C).

XPS scans were performed to determine the elemental compositions of Cu/Ls MOF and the oxidation number of Cu ions. Cu/Ls was composed of Cu, O, N and C elements, suggesting that the Cu/Ls were organic-inorganic hybrids (Fig. 3D). The spectra revealed a core region (Cu2p2/3 and Cu2p1/2) along with strong Cu(II) satellite peaks (Fig. 3E). In the Cu 2p XPS spectra, the peaks of 933.7 and 954.8 eV were characteristic of Cu(II) 2p3/2 and Cu(II) 2p1/2. Two strong satellite peaks also verified that Cu(II) was in a paramagnetic chemical state (Ma et al., 2020). Peaks at lower binding energy at 931.5 and 951.4 eV are attributed to lower oxidation states of Cu (Cu^0 or Cu^+) (Ma et al., 2020) (Yan et al., 2020). Moreover, the Auger Cu LMM spectrum showed the oxidation states of Cu in Cu/Ls MOF and confirmed the presence of Cu^+ and Cu^{2+} assigned to 572.9 eV and 568.2 eV, respectively (Fig. 3F).

3.4 Toxicity assessment of Cu/Ls MOF in *C. elegans*

MOF and other nanoparticles usually exhibit biological toxicity (Kumar et al., 2019). To assess the toxicity of Cu/Ls MOF, N2 worms pretreated with Cu/Ls at concentrations ranging from 0 to 100 $\mu g \cdot mL^{-1}$ in 96-well plates at 20°C. After 24, 48, and 72 h exposure from L4 stage, there was no difference in *C. elegans* survival between the control and Cu/Ls groups. These results indicated that 100 $\mu g \cdot mL^{-1}$ Cu/Ls MOF was safe, which was a high concentration relative to other environmental pollutants. and the acute exposure can be considered as nonlethal in *C. elegans* (Fig. 4A). This concentration was considered for further toxicity studies. Some MOF usually released Cu in the environment (Guan et al., 2021), resulting in Cu ion accumulation in organisms and the possible promotion of oxidative stress. Excess of ROS cause death (Vaccaro et al., 2020) and other adverse effects, such as growth and development (Palozza et al., 2011). The result demonstrated that the ROS levels increased when exposing *C. elegans* to Cu/Ls MOF at concentrations of 100 $\mu g \cdot mL^{-1}$ (Fig. 4B).

3.5 Inhibition on body length and locomotion behavior

Body length was used to assess the growth and development of *C. elegans* (Li et al., 2021). The L1 stage nematodes were exposed to 100 $\mu g \cdot mL^{-1}$ of Cu/Ls MOF for 24, 48, and 72 h in K-medium. After 48 h of exposure, the nematodes pretreated with Cu/Ls MOF showed a significant decrease in body length compared to the control group (Fig. 4C). The developmental retardation was more severe in nematodes exposed to Cu/Ls MOF for 72 h. (Fig. 4C), where the *C. elegans* seem to stop growing. These results indicated that 100 $\mu g \cdot mL^{-1}$ Cu/Ls MOF could induce a considerable disturbance on development.

Body bends was used to assess the effect on the motor behavior or muscle function of *C. elegans* (Xiao et al., 2019). At a 24 h exposure of *C. elegans* L4 stage to Cu/Ls MOF in K-medium, a significant reduction in body bends of the nematode was detected. (Fig. 4D). This result indicated that 100 $\mu g \cdot mL^{-1}$ Cu/Ls MOF could induce a decrease of exercise activity.

3.6 Effect of Cu/Ls on the feeding ability and reproduction of *C. elegans*

The feeding of *C. elegans* was determined based on the change in pharyngeal pumping (Calahorro et al., 2019). Similarly, for worms exposed to 100 $\mu g \cdot mL^{-1}$ of Cu/Ls MOF in NGM, the feeding ability was not altered (Fig. 4D). These results suggested that 100 $\mu g \cdot mL^{-1}$ Cu/Ls did not cause any toxic effect on the reproduction and feeding ability of *C. elegans*.

To examine the effect of Cu/Ls MOF on reproductive function, the L4 stage nematodes were exposed to 100 $\mu g \cdot mL^{-1}$ of Cu/Ls MOF in NGM until the end of their breeding period. There were no significant effects on the reproduction of *C. elegans* after exposure to Cu/Ls MOF (Fig. 4E).

Conclusions

In this paper, we documented the synthesis and functional characterization of Cu/Ls MOF and their effects on an animal model. Cu/Ls MOF was synthesized by a simple synthetic method and displayed laccase-like catalytic activity. According to the SEM and FTIR results, Cu/Ls MOF exhibited a stable micelle structure and stable binding. The characterization and catalytic results implied that a similar phenomenon might occur in discharged domestic sewage in nature with the possible capability to oxidize phenolic compounds. In addition, we demonstrated that Cu/Ls MOF do not exhibit any effects regarding acute toxicity or reproductive toxicity on *C. elegans*, but a reduction in animal exercise viability and an inhibition of larval growth can be observed. The raw materials of Cu/LS MOF are everywhere, especially in detergents, and may provide a safe alternative to treatment of environmental pollutants. The outline of the article is shown in Fig 5.

Declarations

Ethical Approval: Not applicable.

Availability of data and materials: Not applicable.

Consent to Participate: Not applicable.
Consent to Publish: Not applicable.

Conflicts of interest: There are no conflicts to declare.

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Authors Contributions: Conceived and designed the experiments: Hui Li, Feng Guo and Zhendong Fu; performed the experiments: Hui Li, Feng Guo and Zhendong Fu; analyzed the data: Hui Li, Feng Guo and Zhendong Fu; contributed reagents/materials: Hui Li and Feng Guo; contributed to the writing of the manuscript: Hui Li; Review & editing, Liping Wang.

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Figures

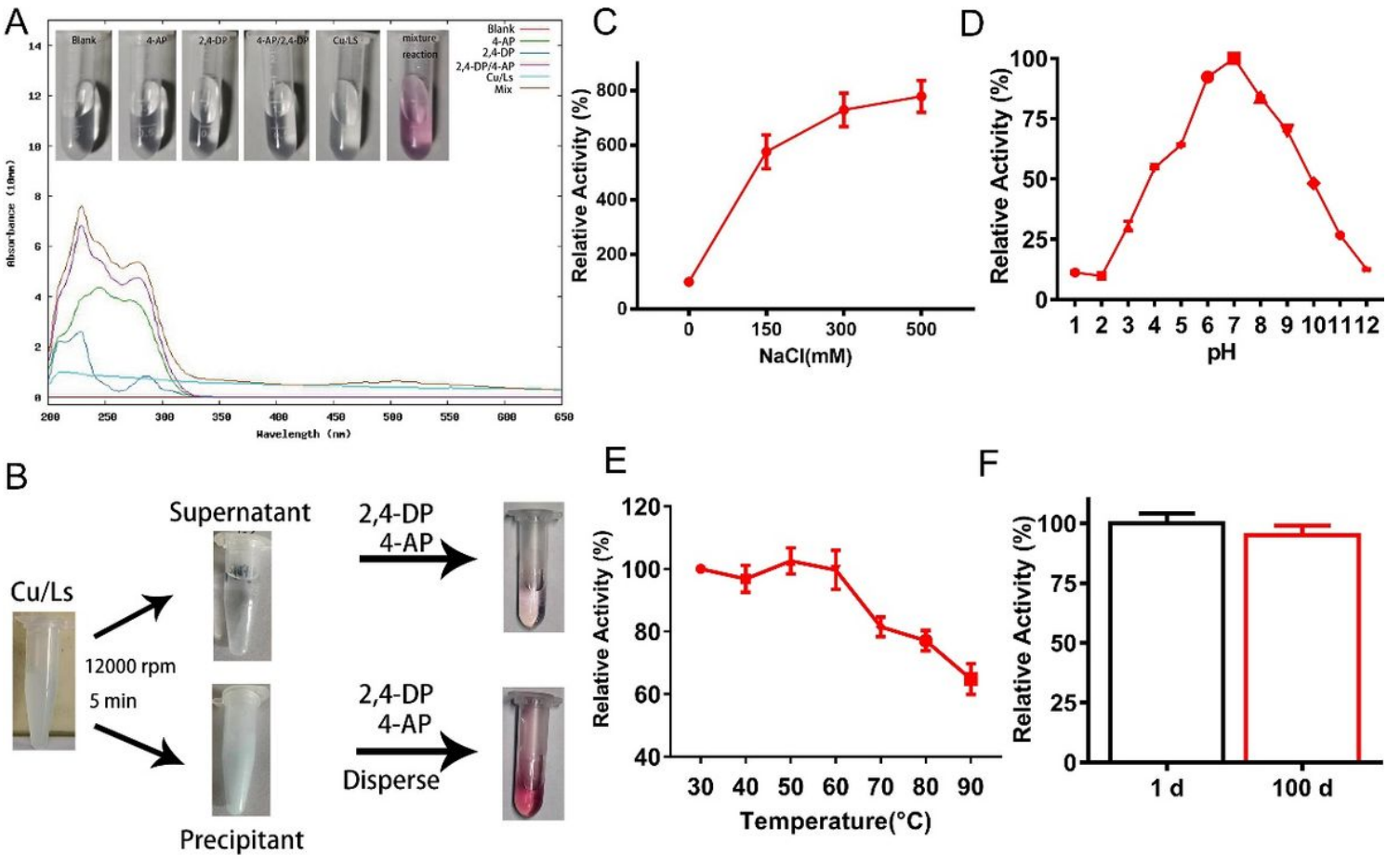

Figure 1

(A) UV–vis spectra of the three substrates and their reaction product. Mix: (B) Cu/Ls MOF reacted with 2,4-DP and 4-AP in pH 7.0 MES buffer after centrifugation. The catalytic activity belonged to Cu/Ls MOF. Stability of the Cu/Ls at different (C) NaCl concentration, (D) pH, (D) temperature. (F) Long-term stability of Cu/Ls.

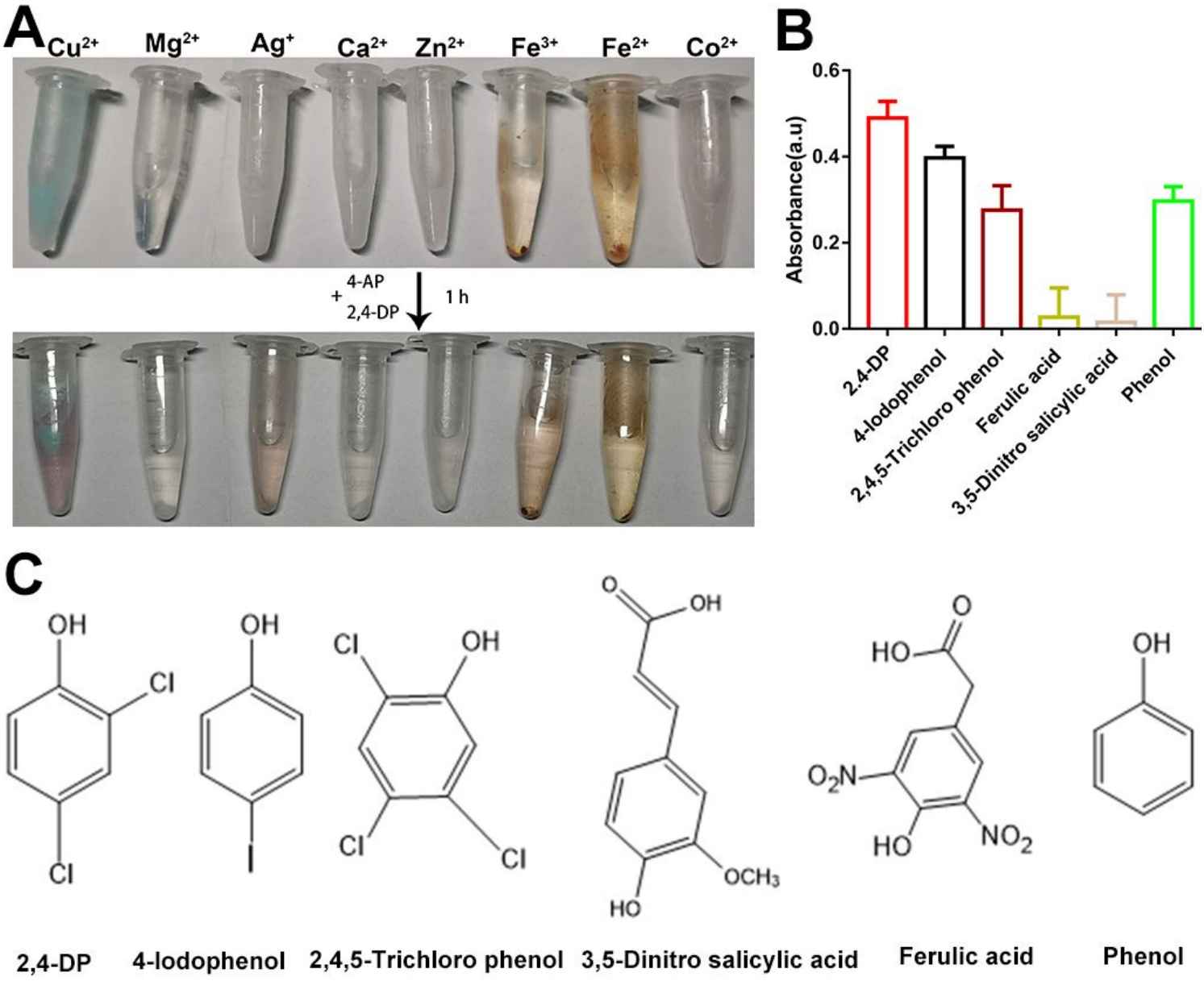

Figure 2

(A) Precipitates formed by Ls with different metal ions (top) and color change of the reaction system after the addition of the precipitates (below). (B) The ability of Cu/Ls MOF to catalyze different phenolic substrates. (C) the structure of phenolic substrates.

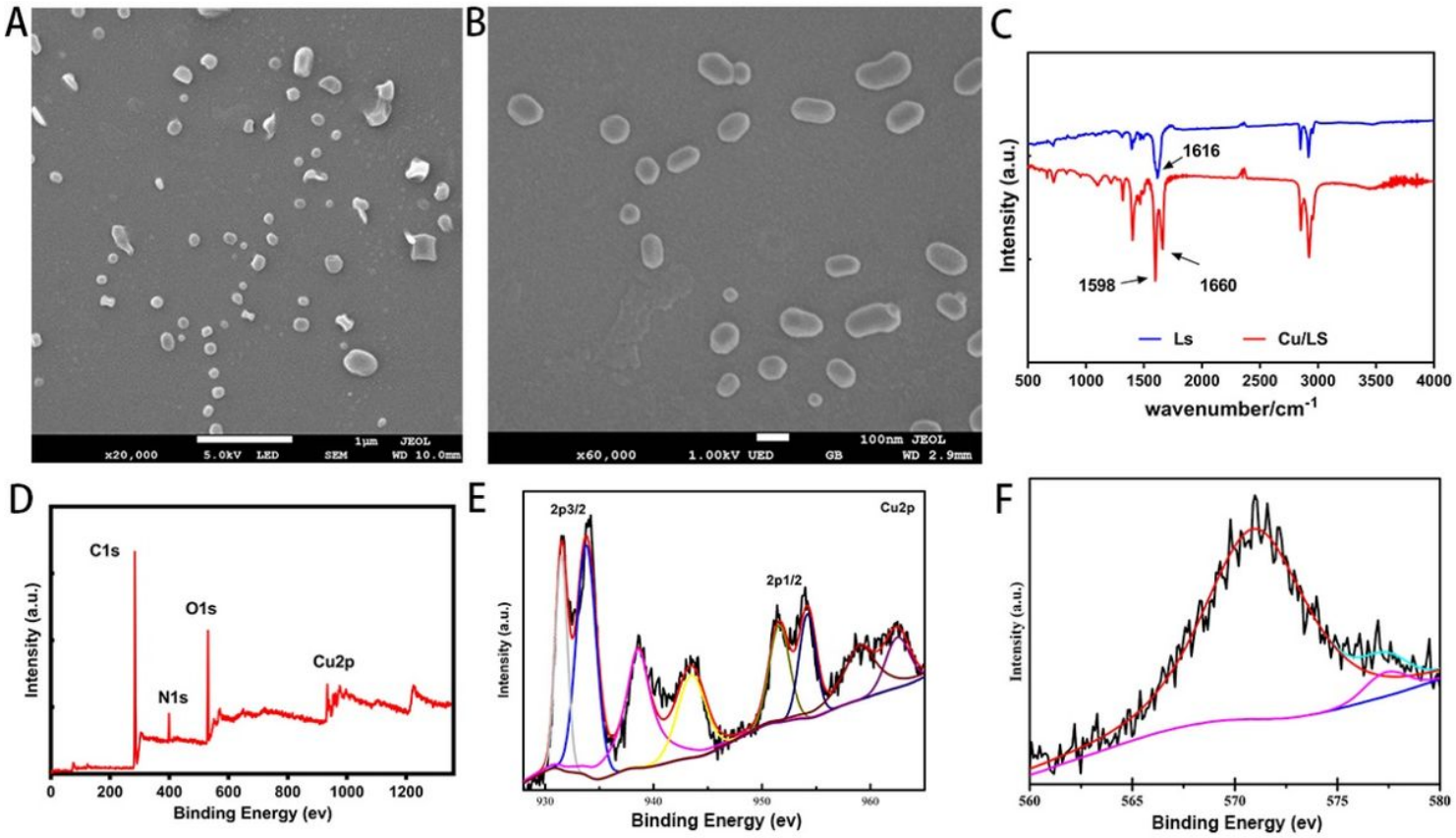

Figure 3

(A) and (B) SEM images showing the surface micrographs of Cu/Ls at different magnifications. (C) FTIR spectra (500–4000 cm−1) of Ls and Cu/Ls MOF. (D) XPS fully scanned spectrum for different elements in Cu/Ls MOF. (E) XPS spectrum of Cu2p. (F) The Auger Cu LMM spectrum of Cu/Ls MOF.

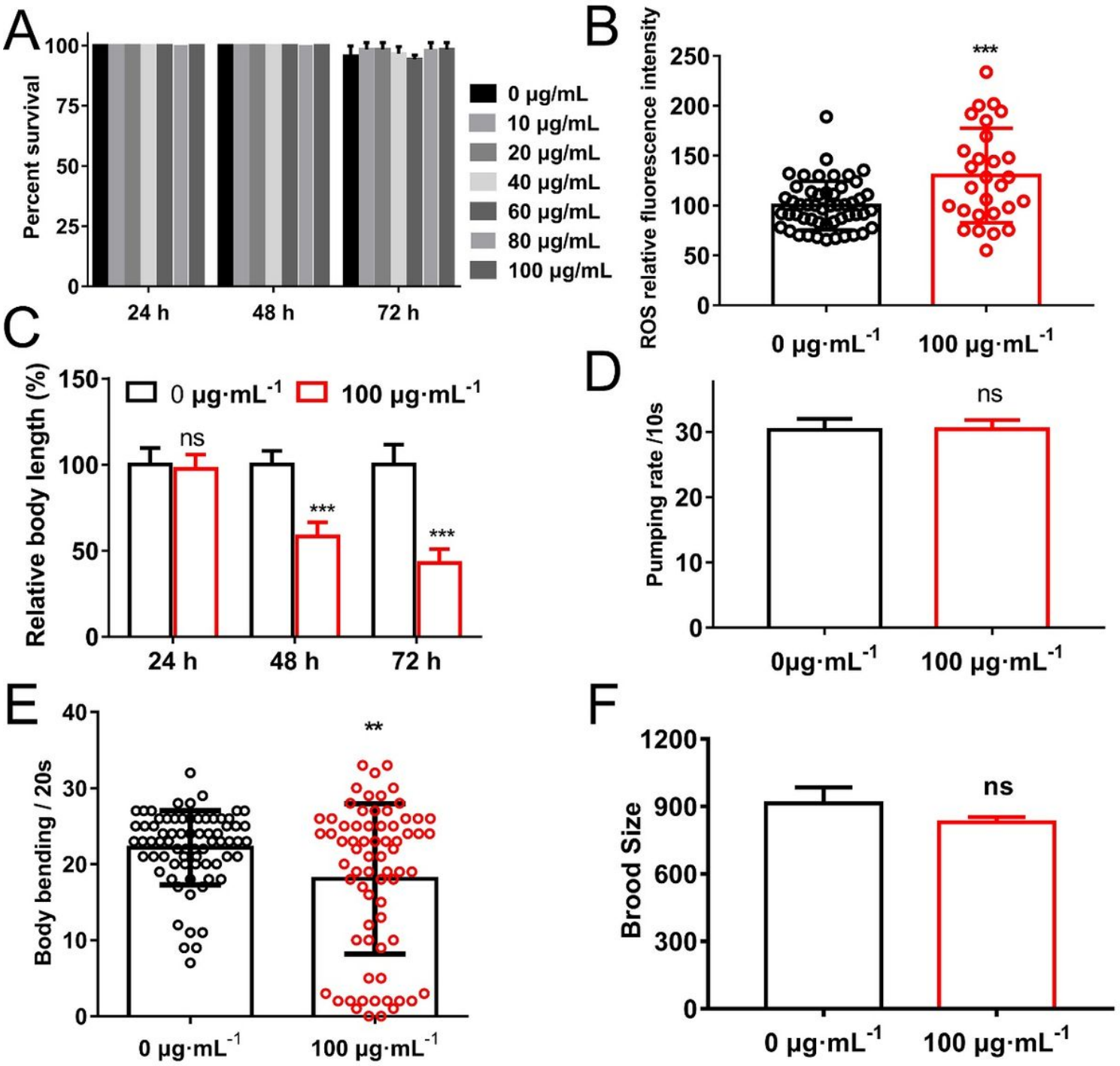

Figure 4

Effect of exposure to Cu/Ls MOF on C. elegans. (A) Survival of N2 C. elegans pretreated with or without Cu/Ls MOF for 24, 48, and 72h. (B) Effects of Cu/Ls on ROS levels in N2 C. elegans. The effect of exposure to Cu/Ls MOF on C. elegans physiological parameters: (C) body length (~30); (D) body bending (~70); and (E) reproduction (n=3); (F) pumping rate (n=10).

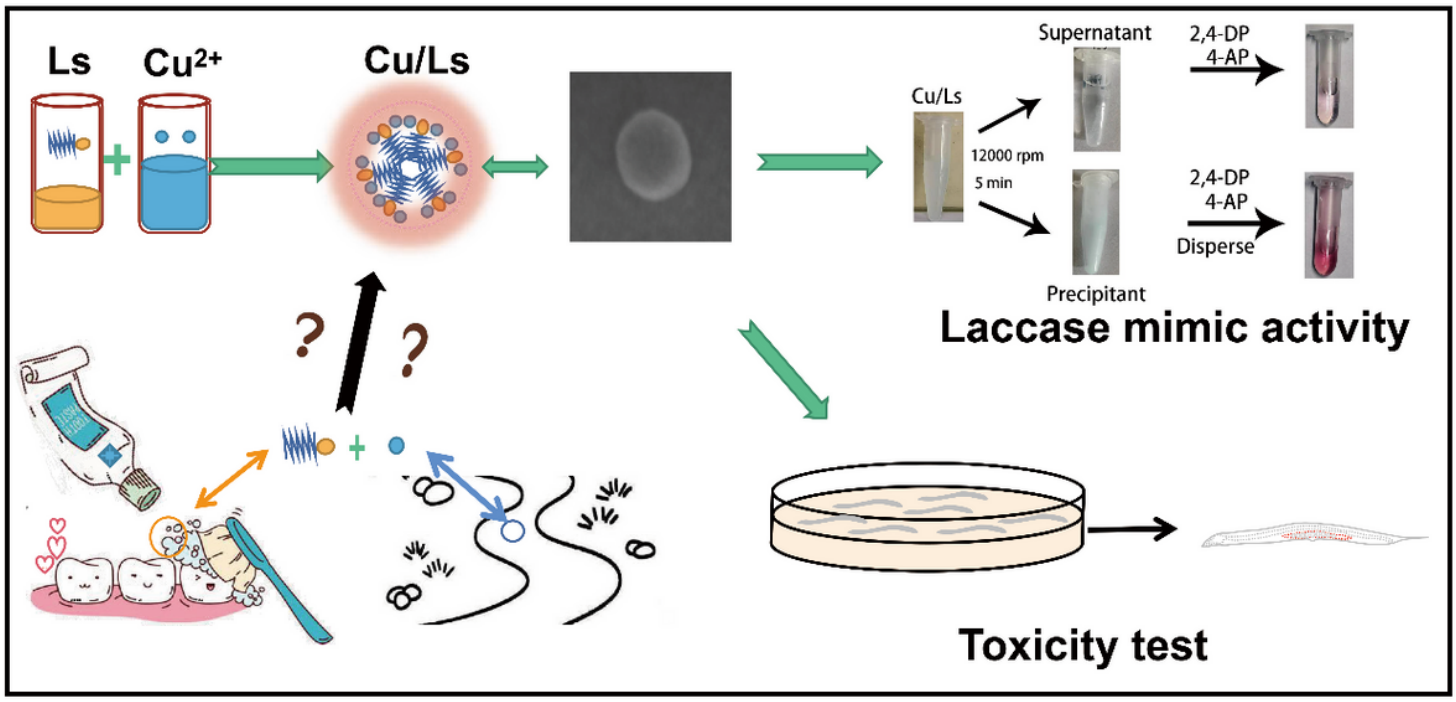

Figure 5

Ls and Cu2+ formed a stable complex (Cu/Ls MOF), which can oxidize phenols. C. elegans were used to detect the toxicity of Cu/Ls MOF.

Supplementary Files

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