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Mycoremediation potential of *Coprinus comatus* in soils co-contaminated with copper and naphthalene

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Abstract

The experiment was carried out to investigate the effects of mycoremediation by *Coprinus comatus* (*C. comatus*) on biochemical properties and lettuce growth in copper and naphthalene (Nap) co-contaminated soil. Results showed a significant enhancement on Nap dissipation incubated with *C. comatus*, and the removal ratios ranged from 96.00 to 97.16% with the level of contaminates, which were associated with the production of ligninolytic enzymes. The accumulation of copper in the body of *C. comatus* showed a positive correlation with augment of metal loaded, and the proportion of acetic acid extractable copper in unplanted soils was larger than in soils with *C. comatus*. Lettuce grown in bioremediated soils showed higher biomass and germination percentage and lower copper uptake than in non-bioremediated soils. These results suggested that the accumulation of copper and degradation of Nap by *C. comatus* provide a candidate for the bioremediation in sites containing multiple pollutants.

Key words: *Coprinus comatus*; Lettuce; Copper; Naphthalene; Co-contamination

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Introduction

Heavy metals and PAHs released into the environment have serious threaten to natural ecosystems and public health due to their toxicity and bioaccumulation.[1-3] Due to long-term anthropogenic activities, Cu concentration in soils by repeated application of Cu salt as fungicides can reach values 10-100-fold larger than in non-contaminated soils, and building excessive Cu concentration in topsoil have affected plant communities and plant performances.[4-6] Naphthalene (Nap) has the lowest molecular weight among the sixteen PAHs listed as priority pollutants by the United State Environmental Protect Agency (USEPA) and a high concentration of Nap is commonly found in both aqueous and solid phases in the environment.[7-9] Among multiple metal and organic polluted sites, co-contamination of Cu and Nap often occurred in soil environments as a result of wastewater irrigation, solid waste disposal, and industrial activities.[10] Moreover, remediation of sites co-contaminated by metal and organic pollutants is a very complex problem, since the chemical processes and remediation technologies are different for each group of pollutants. Therefore, it is critical to develop a cost-effective and eco-friendly technology to remove heavy metals and PAHs from co-contaminated soils.

In recent years, phytoremediation has received considerable attention to assimilate, metabolize, detoxify or degrade metal and organic chemical contamination.[11-13] However, there are many limitations in hypertoremediation. For example, hyperaccumulators are generally small and grow slowly, making them difficult to accumulate a mass of pollutants.[14] In addition, because of the lack of PAHs degradation capacity, the

dissipation of pollutants by growing hyperaccumulators enhanced very slightly.[15]

Compared with hyperaccumulators, mushroom which has big biomass and grows fast has been cultivated all over the world.[16] Up to now, there are numerous promising results indicating that mushroom has a high accumulation for heavy metals including Cadmium, Lead, Copper, etc.[14, 17] Meanwhile, previous studies has illuminated that mushroom has the capacity to degrade organic compounds on account of the production of ligninolytic enzymes.[18, 19] Therefore, mushroom possess a more effective mechanism than plants to remediate heavy metal and organic co-contaminated soils.

Coprinus comatus is a white rot basidiomycete with high contents of proteins and has an excellent performance in producing ligninolytic enzymes.[20] However, little information is available on effectiveness of mycoremediation concerning heavy metal and organic pollutants, especially about remediation by *C. comatus* for co-contaminated soils of heavy metals and organics. The aim of this study was to investigate the influence of co-contamination on the growth of *C. comatus* and the fate of pollutants in soil and mushroom. After *C. comatus* being harvested, lettuce (*Lactuca sativa* L.) was used to test the effect of bioremediation as a large number of studies have indicated that massive plants could accumulate heavy metals and organics, and the toxicity of pollutants had serious effect on the growth of plants.[21, 22] Several researchers has been demonstrated that lettuce growth would be inhibited in soils contaminated with heavy metals.[23, 24] Hence, the growth response and heavy metals accumulation of lettuce could further evaluate the remediation performance.

2. Materials and methods

2.1 Soil preparation

Soil samples used for in this study were collected from campus area with pH 7.12, 1.68% organic matter, originally free of Nap and 26 mg Cu kg^{-1} soil in Sichuan University, Chengdu, China. Soil samples were air dried and sieved through a 2 mm mesh and then carefully weighed for spiking with heavy metal and organic pollutants. The levels (mg kg^{-1}) of Cu and Nap added into the soil were T0 (Cu0 + Nap0), T1 (Nap250), T2 (Nap500), T3 (Cu100), T4 (Cu200), T5 (Cu300), T6 (Cu100 + Nap250), T7 (Cu100 + Nap500), T8 (Cu200 + Nap250), T9 (Cu200 + Nap500), T10 (Cu300 + Nap250), T11 (Cu300 + Nap500), including the planted and unplanted groups with three replicates. Briefly, the bulk soil was first mixed with Nap by dissolving in acetone. Then solutions of Cu (as $CuCl_2$) with different concentrations were added into Nap-spiked soils. After the acetone had evaporated, the spiked soils were sieved again through a 2 mm mesh and packed into pots (2 kg dry weigh soil per pot), then covered with aluminum foil, and equilibrated in the dark room for two months prior to the experiment.

2.2 Pot experiments

This experiment was carried out in clean plastic pots (height 9 cm, diameter 12 cm) containing 2 kg of above contaminated soil and 0.1 kg of the mycelia bag of *C. comatus* bought from Shuangliu, Chengdu, China. In three replicates, the soil was wetted with deionized water three times a week to approximately 65% soil field water capacity. At the bottom of each pot, there was a plastic dish to collect any potential leachate. After about

60 days, the mature fruiting bodies were harvested from the pots, washed with deionized water and dried for 4 days at 60 °C in oven.

After *C. comatus* being harvested, soil of each pot was collected carefully and air-dried and then sieved through a 3 mm mesh again for lettuce experiment. Each pot was sowed with thirty seeds of lettuce, then wetted with deionized water everyday. After 30 days, the lettuce was harvested, washed with deionized water and dried for 2 days at 60 °C in oven.

2.3 Soil analysis

Soil samples of cropped and uncropped *C. comatus* were collected at harvest, oven dried at 80 °C for three days and BCR sequential extraction procedure was applied for metal speciation according to Quevauviller et al. with some modification.[25] Briefly, 1.0 g of soil were shaken at 25 °C, 250 rpm for 16 h with 40 mL of 0.11 M CH_3COOH, then centrifuged for 5 min with 8000 r min^{-1} and the supernatant were collected for assay the acetic acid extraction state. For the combined with oxidation state, the above residue was shaken at 25 °C, 250 rpm for 16 h with 40 mL mixture of 0.5 M $NH_2OH{\cdot}HCl$ and 0.05 M HNO_3, then also centrifuged for 5 min with 8000 r min^{-1} and the supernatant were collected for assay. For organic combination of state, the above residue was added with 10 mL 30% H_2O_2 (pH = 2.5), kept in a bath at 85 °C for about 1 h and till the volume of liquid was less than 3 mL, then the residue was extracted again with 10 mL 30% H_2O_2 and the volume of liquid was less than 1 mL, finally adding 50 mL 1.0 M CH_3COONH_4 (pH = 2) and centrifuging for assay. For the residual fraction, the above residual soil was

digested with the mixture of 6 mL HNO_3, 4 mL $HClO_4$, and 3 mL HF using a microwave digestion method to extract the residual fraction. All fractions of Cu in samples were determined by flame atomic absorption spectrometry (AAS; VARIAN, SpecterAA-220Fs).

Extraction of Nap from soil was performed following the method described by Huang et al with some modification.[26] Concentrations of Nap were determined by HPLC with a UV-Vis detector, operating at a wavelength of 254 nm and a reverse phase 5 μm C-18 column (250 × 4.6 mm). The mobile phase used was acetonitrile-water (90:10, v/v) at a flow rate of 0.6 mL min^{-1}.

2.4 Analysis of mushroom

C. comatus was harvested once the fruit bodies unfolded and washed with deionized water three times. The fresh samples (0.5 g) were quickly frozen in liquid nitrogen and grinded by a precooled mortar and pestle, and then extracted in 5 mL of 200 mM phosphate buffer (pH 7.8) at 4 °C. The homogenate was centrifuged at 5500 rpm for 30 min and the obtained supernant was used for measuring the soluble protein and ligninolytic enzymes. Soluble protein content in *C. comatus* was measured using bovine serum albumin as the standard protein.[27] Laccase activity was measured as described by Palmieri et al. with one unit of laccase activity was defined as the amount of enzyme that catalyzed the oxidation of 3-ethylbenzothiazolone-6-sulfonic acid (ABTS) at 30 °C in 1min.[28] Manganese peroxidase (MnP) activity was measured according to Lopez et al. with one unit of enzyme activity was defined as the amount of the enzyme which can

produce 1 μM Mn^{3+} from the oxidation of Mn^{2+} per minute.[29] Lignin peroxidase (LiP) activity was measured as described by Tien et al. with one unit of LiP was defined as 1 μM of veratryl alcohol (VA) oxidized to veratraldehyde per minute.[30] After the fruit bodies of *C. comatus* were oven-dired, samples (0.1 g) of mushroom powder were digested with the mixture of 3 mL HNO_3, 1 mL 30% H_2O_2 and 1 mL HF at microwave and then diluted to 10 mL with deionized water. Finally, the concentrations of Cu in *C. comatus* were determined by FAAS.

2.5 Analysis of lettuce

The germination percentage was recorded at two weeks after lettuce seeds were sowed and no seeds sprouted afterward. After about 30 days, lettuce was harvested, washed with deionized water three times, and dried at 65 °C for two days to determine the dry weight and the content of heavy metal. The concentration of Cu in lettuce was measured as the same as the determination of Cu in mushroom.

2.6 Date analysis

Translation factors (TF) values of metal from soils to mushrooms were calculated according to the formula: $TF = \frac{\text{Metal concentration in cap}}{\text{Metal concentration in stipe}}$

The percentage of TCP removal from soils was calculated as: Nap removal rate (%) =

$$\frac{\text{Initial concentration of Nap in soil} - \text{Concentration of Nap in soil after harvest}}{\text{Initial concentration of Nap in soil}}$$

All treatments were replicated three times in this experiment. Treatment means were evaluated using variance and the Tukey's test ($p < 0.05$). Statistical analysis was carried

145 out using SPSS 18.0.

146 **3 Results and discussion**

147 **3.1 Mushroom growth**

148 The growth of *C. comatus* was significantly affected by Cu, PAHs and their
149 interactions. As the Table 1 shows, the addition of Cu could facilitate the growth of *C.*
150 *comatus* under a low level (T3), resulting in an increase at the rate of 18.25% when
151 compared with the control group (T0). However, *C. comatus* showed visual signs of
152 toxicity in response to single Nap contamination and to mixed contaminants, and total
153 biomass significantly decreased by 19.24 and 22.86% in the high Cu treatments (T4 and
154 T5). Furthermore, it was observed that an addition of Nap further decreased the biomass
155 of *C. comatus* in Cu treatments compared to the Cu treatment alone. The highest decrease
156 occurred in the 200 and 300 mg Cu kg^{-1} with Nap compared without, whereas no
157 significant difference was observed detected in the other treatments.

158 The present study clearly demonstrated that contaminants of heavy metal and PAHs
159 had a direct effect on biomass production, and Nap showed a stronger toxicity than Cu.
160 Similar to our study, Chigbo et al. suggested that pyrene had a strong inhibition on
161 *Brassica juncea* than Cu, and the increased growth in low Cu concentration could related
162 to the effects Cu has on various macronutrient contents (N, P, K, Na, Mg).[31] Zhang et al.
163 showed that the interaction of Cd and PAHs caused a stronger inhibition on *Juncus*
164 *subsecundus* than Cd or PAHs alone.[32] Our results, however, are different with the report
165 by Zhang et al. which showed that pyrene did not alleviate the toxicity Cd to *Z. mays*.[15]

These results suggest that growth response to joint toxicity of metal and organic depend on certain factors including species and characteristics of pollutants.

Although, growth was inhibited under some treatments of Cu and Nap, *C.comatus* performed excellent tolerance to toxicity stress and confirmed a potential ability to remediate metal and PAHs co-contaminated soil.

3.2 Mushroom soluble protein content and enzyme activities

Soluble protein content and enzyme activities in mushroom were measured after 60 days incubation**.** Soluble protein in *C. comatus* decreased from 27.52 to 73.39% for Cu (T3-T5) and from 29.81 to 45.11% for Nap (T1-T2) compared to control (Fig. 1), which showed that contaminants with Cu and Nap could effectively induce the protein content in the bodies of *C. comatus*. Moreover, it was obvious that the co-effects of Cu and Nap led to an induction of protein content in *C. comatus.* When 300 mg Cu kg^{-1} was mixed with 500 mg Nap kg^{-1} (T11), the protein decrease reached maximum, about 645.79% lower than control.

Laccase and Lip activities (Fig. 1) in the *C. comatus* represented significant increase under joint stress of Cu and Nap in comparison with control. In the same concentration of Nap, laccase and Lip activities tended to increase with increasing level of Nap from 0 to 500 mg kg^{-1} in soil. The maximum laccase and Lip activities were observed in the T11 and T9, which were 316.09% and 240.49% higher than control, respectively.

Mnp activity was more complex than laccase activity and Lip activity (Fig. 1) and

reached maximum in the 250 mg Nap kg^{-1} mixed with 200 mg Cu kg^{-1} (T8), about 232.15% higher than control. As the figure shows, the activity of Mnp increased in the level of 250 mg Nap kg^{-1} in comparison with the soil spiked with Cu of 0, 100, 200 and 300 mg kg^{-1} alone, especially in the 200 mg Cu kg^{-1}. In the same concentration of Nap, however, Mnp activity showed no significantly difference ($p < 0.01$) at the 100 and 300 mg Cu kg^{-1} compared to control.

Heavy metal and PAHs are known to increase the activities of ligninolytic enzymes (laccase, Mnp, and Lip), which can partly reduce the toxicity stress and degrade organic compounds.[33, 34] In *Pleurotus ostreatus*, addition of Cu (0.5-5mM) or Cd (1-5 mM) could not only induces laccase by the expression of laccase genes, but also positively affects the activity and stability of the enzyme.[35] These results presented in our study proved that the secretion of ligninolytic enzymes in *C. comatus* could be enhanced in co-contaminants, attesting the potential removal of PAHs.

3.3 Cu accumulation and translocation in mushroom

The metal accumulation and translocation in the fruiting bodies of *C. comatus* were significantly influenced by the concentration of Cu, PAHs, and their interactions (Fig. 2). Cu concentration in cap and stipe of *C. comatus* tended to increase with increasing Cu amounts in soils, and was 8.21-103.7 mg kg^{-1} and 5.58-65.2 mg kg^{-1} across all the treatments, respectively. Comparison of Cu-alone and Cu-Nap contamination indicated that the addition of Nap could increase the accumulation of Cu in cap (except in the T8) and in stipe (except in the T8 and T10). Especially in the T11, the accumulation of Cu in

208 cap and stipe reached to maximum, about 128.50% and 116.34% higher than in the T5.
209 Some previous reports also have shown that the interaction between metals and PAHs
210 could influence metal uptake and accumulation in co-contaminated soil. Increased Zn
211 concentrations were found in shoots of Indian mustard (*Brassica juncea*) grown in soils
212 contaminated with a mixture of pyrene and Zn.[36] The PAHs increased Cu uptake by a salt
213 marsh plant (*Halimione portulacoides*) in elutriate, but not in the presence of
214 sediments.[37] However, Lin et al. found that the ability of Cu phytoextration of *zea mays L.*
215 would be inhibited under the Cu-Pyr co-contaminated soil.[21] Chen et al. observed a slight
216 decrease in the accumulation of Cu in *Lolium perenne* in Cu-2,4-dichlorophenol
217 co-contaminated soil.[38] Furthermore, it was also observed that accumulation of Cu was
218 higher in cap than that in stipe, which agreed with these reports.[17, 39, 40] In the absence of
219 Nap, the results of TF values first significantly increased in 100 mg Cu kg^{-1} (T3), then
220 decreased in 200 and 300 mg Cu kg^{-1} (T4 and T5), about 133.57%, 100.95% and
221 102.14% higher than control, respectively. When soil co-contaminated with Cu and Nap,
222 however, Nap influenced Cu concentration and accumulation, which depends on the
223 various levels of Cu treatment. For example, in 100 and 200 mg Cu kg^{-1} soil, TF values
224 first decreased in 250 mg Nap kg^{-1}, then increased in 500 mg Nap kg^{-1} and even reached
225 2.07 in T7. But in high dose of Cu (300 mg Cu kg^{-1}), TF values first significantly
226 increased in 250 mg Nap kg^{-1}, then decreased in 500 mg Nap kg^{-1}. That is to say, in lower
227 Cu-polluted soil, high Nap would increase the translocation of Cu, and in highly
228 Cu-polluted soil, low Nap would decrease the translocation of Cu. Our results could be

229 explained by Alkio et al, PAHs may passively penetrate the stipe cell membranes without
230 any carrier which can therefore facilitate the penetration of metal or metal complexes into
231 the cell, which increased the metal in cap.[41] Moreover, Due to their complex interactions
232 of PAHs and metal in soil, the translocation efficiency of Cu would be influenced by their
233 different concentrations in varying degrees.

234 **3.4 Cu speciation in soil**

235 For phytoremediation or mycoremediation, Cu must be bioavailible, which suggests
236 metal accumulation in mushroom is dependent on not only their total concentration, but
237 also their chemical forms.[42] To study the distribution of different forms of Cu in the soil,
238 four chemical fractions of Cu in planted and unplanted soils were determined with BCR
239 method and the concentrations are shown in Table 2. On the one hand, it was observed
240 that the HOAc extractable Cu decreased and the immobilized metals were transformed
241 mainly into oxidizable forms in planted soil after 60 days culture compared with
242 unplanted soil in Cu added treatments. The proportion of HOAc extractable Cu in planted
243 soil decreased by 3.08%-20.04% and oxidizable Cu increased by 19.26%-107.18%
244 relative to unplanted soil (Fig. 3), respectively. A possible explanation could be the
245 exchangeable form Cu in planted soil was the predominant species for Cu uptake by
246 mushroom, which was consistent with the result of Cu accumulation in *C. comatus* (Fig.
247 2). Hence, *C. comatus* can significantly decrease the concentration of active and
248 bioavailable heavy metal by its uptake and accelerating the stability process. On the other
249 hand, the proportion of reducible and residual Cu either remained stable or changed only

slightly. Probably, the short incubation time not lead to any marked change in reducible and residual portion of the heavy metal.[43]

3.5 Removal of Nap in soil

The concentrations of Nap in soil after about 60 days were shown in Fig. 4. The residual concentrations of Nap in *C. comatus* planted soil were significantly lower than in the unplanted soil. In 250 and 500 mg Nap kg^{-1} soil, the residual concentrations of Nap in *C. comatus*-planted soils were 7.01-8.12 and 18.07-20.12 mg Nap kg^{-1}, about 27.06%-33.28% and 28.76%-30.10% lower than in unplanted soils, respectively. Furthermore, the removal ratios were elevated in planted soil, and the maximum of removal ratio (97.20%) was observed in T7 compared with treatments (93.41%-94.3%) without the incubation of *C. comatus* (Fig. 4). These results indicated that the removal of Nap was clearly enhanced by planting mushroom. The effect of heavy metal on dissipation of PAHs may be positive or negative, while the presence of Cu showed no-significant effect on the removal of Nap in this study.

The fates of PAHs in spiked soils mainly include volatilization, leaching, plant uptake, biodegradation, photo-degradation, and other abiotic losses.[44] Volatilization, photo-degradation, and microbial activity are most possibly related to the removal of Nap in unplanted soil, and the enhanced removal of Nap in planted soil can be attributed to the phenomena of mushroom uptake and biodegradation. Previous studies have reported that the removal pathway of PAHs in plants, such as *Tall fescue, Tagetes patula, Rumex crispus*.[45-47] The presence of *C. comatus* could product ligninolytic enzymes (Fig. 1) and

lead to a degradation of Nap.

3.6 Lettuce growth responses

Plant growth in response to pollutant is sensitive. The growth response of lettuce and Cu uptake by lettuce are shown in Table 3. Biomass of lettuce was unaffected by residual of Nap because Nap in soils was very rare after remediation. However, biomass of lettuce gradually decreased with increasing concentrations of Cu, which was agreed with the previous reports.[23, 48] Compared with the non-remedied soils, there was a significant increase of biomass in soils after growing *C. comatus*, and the maximum biomass was observed in the T10, about 313.64% higher than in non-remedied soil. In addition, the trend of germination percentage (Table 3) of lettuce was similar as the biomass and the maximum germination percentage in remedied soil was observed in the T11, about 262.47% higher than in non-remedied soil. Moreover, there was a rather straightforward comparison of Cu accumulation in lettuce between non-remedied and remedied soil (Table 3). Planting mushroom significantly decreased Cu accumulation in lettuce and the maximum decrease was 67.58% in the T5, which was consistent with the result of HOAc extractable Cu in soils (Fig. 3). The above results suggested that incubation with *C. comatus* could facilitate the growth, induce the Cu accumulation of lettuce and further confirmed a beneficial remediation effect of mushroom in Cu and Nap co-polluted soil.

Conclusions

There are some conclusions based on this experiment as follows: (1) *C. comatus* was

tolerant to all concentrations of co-contamination and showed potential ability to remove heavy metal from co-contaminated soil (7.03-84.45 mg kg^{-1} for Cu). (2) Planting *C. comatus* facilitated the removal of Nap, and the removal ratios were over 96.0%. (3) The presence of *C. comatus* decreased HOAc extractable Cu (3.08-20.04%) in soil. (5) Activities of ligninolytic enzymes significantly increased when *C. comatus* was exposed to Cu and Nap pollutants, which could be benefit for defensing against Cu and Nap toxicity stress. (6) The effect of remediation with *C. comatus* enhanced biomass and germination percentage of lettuce and significantly decreased the accumulation of Cu. These findings, therefore, provide evidence for the potential mushroom remediation of co-contamination of Cu and Nap with *C. Comatus.*

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Table 1

Biomass (dry weight) of *C. comatus* grown contaminated soils for 60 days.

Treatment	Biomass (g pot^{-1})		
	Total	Cap	Stipe
T0	12.16±1.810c	6.42±0.184f	5.74±1.626e
T1	8.99±4.172ab	4.48±1.365ab	4.50±2.807c
T2	8.50±5.105a	4.24±1.478a	4.26±3.627bc
T3	14.38±3.543d	6.96±2.107g	7.42±1.435f
T4	9.25±0.127ab	5.08±0.085cde	4.17±0.042abc
T5	9.05±4.130ab	5.38±2.510e	3.66±1.619a
T6	9.82±1.336b	4.76±0.205bcd	5.07±1.541d
T7	9.02±2.058ab	4.62±1.775abc	4.40±0.283c
T8	8.83±1.471ab	4.50±0.495ab	4.33±0.976c
T9	9.38±3.988ab	5.22±2.411de	4.16±1.577abc
T10	8.51±2.857a	4.78±0.488bcd	3.73±2.369ab
T11	8.51±1.577a	4.14±1.892a	4.37±0.764c

Results are expressed as means ± SD (n = 3). Date within columns with different letters indicate a significant difference (Tukey HSD $p < 0.05$).

Table 2

Concentrations of different species of Cu in planted and unplanted *C. comatus* soils

Treatment	HOAc soluble-Cu (mg kg^{-1})		Reducible-Cu (mg kg^{-1})		Oxidizable-Cu (mg kg^{-1})		Residual-Cu (mg kg^{-1})	
	planted	unplanted	planted	unplanted	planted	unplanted	planted	unplanted
T0	0.08a	0.00a	5.48a	5.92a	5.22a	5.06a	8.65a	7.20a
T1	0.96a	0.76a	4.42a	6.16a	4.34a	4.07a	8.68a	7.66a
T2	1.48a	1.20a	3.92a	4.40a	4.34a	4.78a	9.15ab	8.24a
T3	31.16b	48.04c	59.56c	56.08b	14.24b	11.82b	10.62bc	11.29bc
T4	38.36b	50.80c	64.36c	57.60b	15.03b	12.37b	12.56de	11.16bc
T5	32.40b	34.84b	31.92b	52.40b	14.75b	13.09b	18.28f	11.22bc
T6	54.96c	64.48d	88.88e	83.24c	22.11c	15.02c	13.13de	12.11bcd
T7	97.64d	99.16e	99.60f	105.96d	26.24def	16.44cd	13.93e	12.34bcd
T8	119.04ef	100.08e	78.40d	104.76d	23.60cd	17.93d	13.94e	13.05d
T9	123.96f	151.28f	102.47f	129.96e	24.86de	16.78cd	13.18de	12.68cd
T10	112.76e	140.64f	105.92f	135.31e	28.16f	16.20cd	11.97cd	16.54e
T11	126.39f	155.70f	104.32f	130.45e	26.83ef	15.24c	12.04cde	15.72e

Date within columns with different letters indicate a significant difference (Tukey HSD $p < 0.05$).

Table 3

Biomass (dry weight), germination percentage, and Cu concentration of lettuce fed in non-remedied and remedied soils

Treatment	Biomass (g)		Germination percentage (%)		Cu concentration in lettuce (mg kg^{-1})	
	Non-remedied	remedied	Non-remedied	remedied	Non-remedied	remedied
T0	0.71±0.03fg	0.78±0.04de	95.00±12.0h	93.33±10.6cd	10.20±1.2ab	9.68±0.6a
T1	0.76±0.05g	0.70±0.02bc	93.33±8.5h	90.00±9.8bc	9.73±0.8a	9.27±0.6a
T2	0.70±0.03ef	0.72±0.03bc	95.00±11.5h	91.67±9.8bc	12.47±1.3b	10.89±1.2ab
T3	0.61±0.02de	0.73±0.03cd	83.33±9.8fg	95.00±11.5d	42.07±3.7d	15.34±1.4de
T4	0.67±0.03ef	0.80±0.05e	86.67±10.6g	90.00±10.5bc	33.69±2.8c	12.13±1.0bc
T5	0.62±0.04def	0.78±0.04de	80.00±8.5f	91.67±11.5bc	40.12±3.9d	13.65±1.4cd
T6	0.56±0.03cd	0.69±0.02bc	66.67±7.4d	88.33±8.5bc	46.22±4.1ef	14.27±1.4cde
T7	0.50±0.02c	0.80±0.02e	73.33±8.0e	93.33±12.0cd	45.83±3.9ef	13.25±1.0bc
T8	0.48±0.01c	0.78±0.03de	70.00±7.2de	86.67±9.2b	45.67±4.3e	15.02±1.6de
T9	0.34±0.02b	0.67±0.01b	46.67±5.88c	66.67±7.8a	48.39±4.7f	15.69±1.3de
T10	0.22±0.01a	0.69±0.02bc	33.33±4.2b	66.67±8.6a	47.40±4.6ef	16.38±1.6e
T11	0.25±0.01a	0.62±0.02a	26.67±2.3a	70.00±7.2a	48.30±4.6f	15.72±1.4de

Results are expressed as means ± SD (n = 3). Date within columns with different letters indicate a significant difference (Tukey HSD $p < 0.05$).

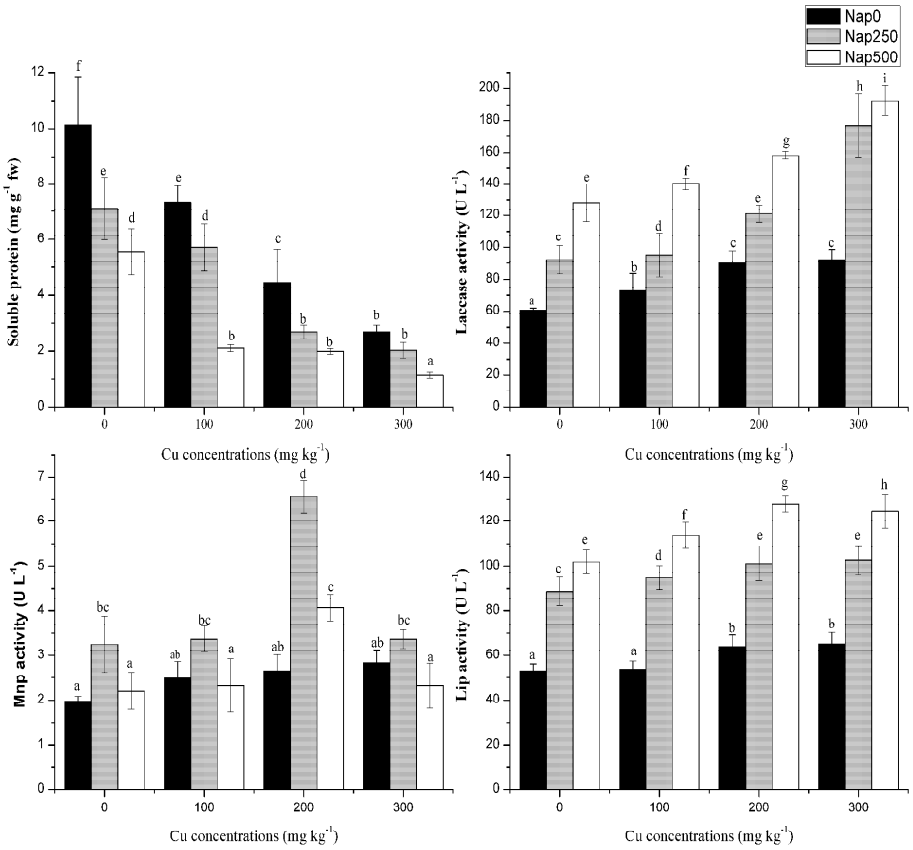
Figure captions

Fig. 1. Concentration of soluble protein and activities of laccase, LiP and MnP in *C. comatus* exposed to different treatments of Cu and Nap. Different letters represent significant differences between the sampling at least $p < 0.05$.

Fig. 2. Cu concentration (column) in *C. comatus* and TF values (line) in treatments with different concentrations of Cu and Nap. Different letters represent significant differences between the sampling at least $p < 0.05$.

Fig. 3. Cu speciation in soil with different concentrations of Cu and Nap.

Fig. 4. Residual concentration (column) and removal rate (line) of Nap in soil with different concentrations of Cu and Nap. Different letters represent significant differences between the sampling at least $p < 0.05$.



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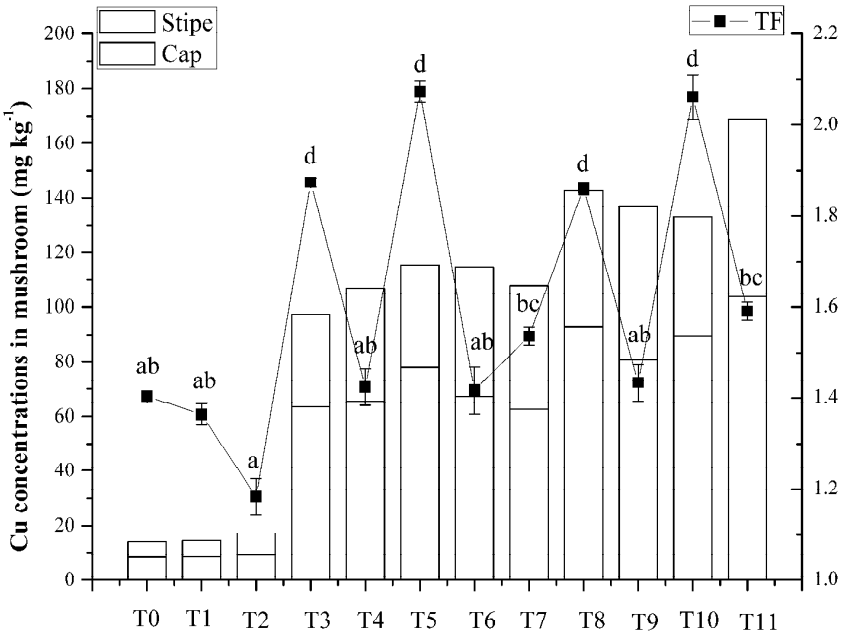

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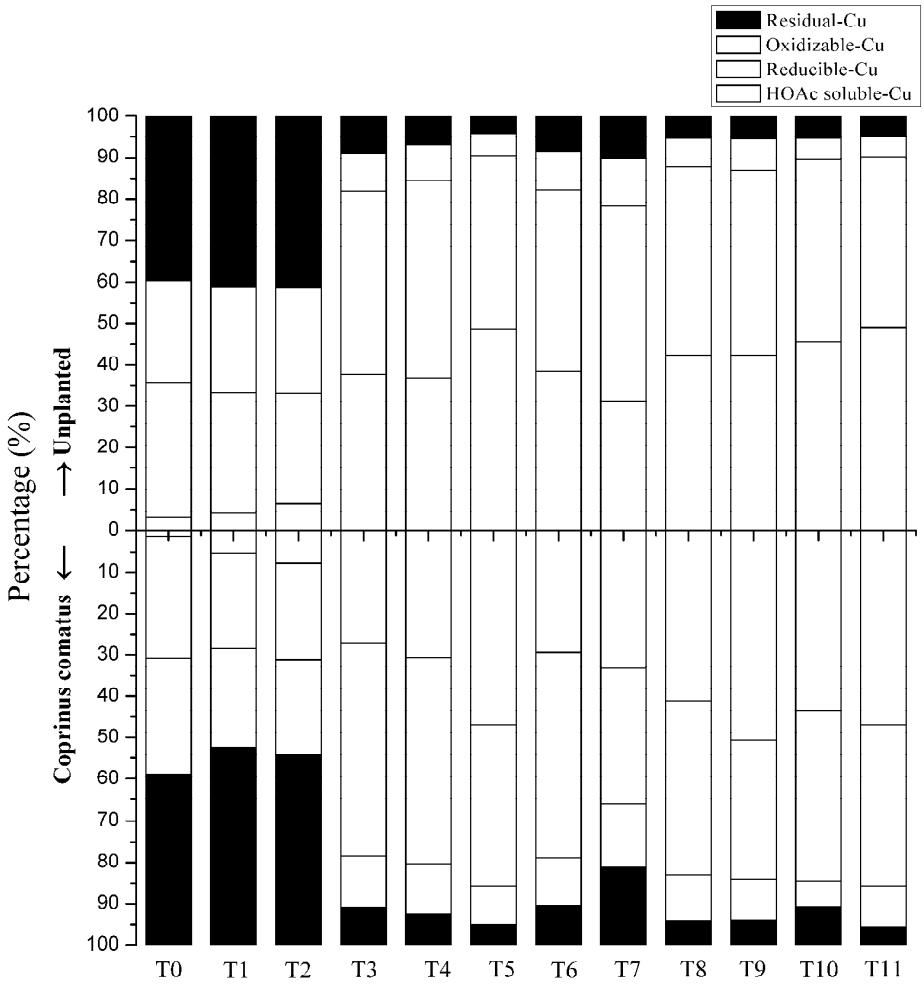

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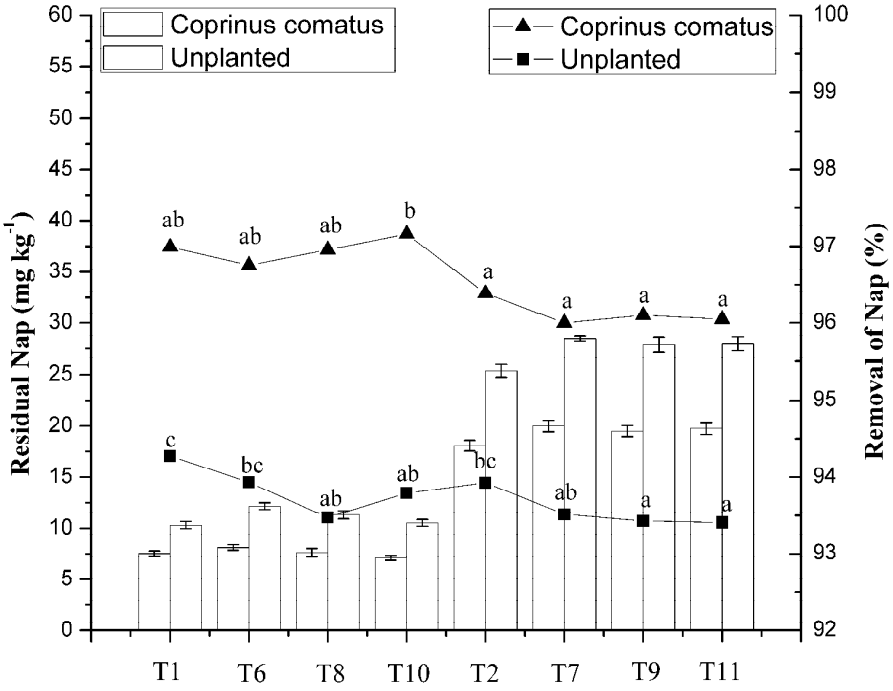

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