In vitro compatibility of fungi for the biosorption of zinc(II) and copper(II) from electroplating effluent

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The present study describes the potential of mixed fungal isolates, i.e. *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizopus oryzae* for the removal of zinc(II) and copper(II) from aquatic environments. Capacity of mixed fungal biomass to adsorb Zn(II) and Cu(II) were studied in batch sorption experiments as bioremediators. Optimal conditions from contact time, pH, initial metal ion concentration and temperature for remediation of Zn(II) and Cu(II) were studied. Typically, the uptake of Zn(II) and Cu(II) rises with increasing pH up to 4.0. Optimal metal concentration was 150 mg/l when the maximum removal of copper and zinc was 69.5% and 30.3% respectively, was observed at initial metal concentration. Maximum uptake for metals was achieved after 15 min. The maximum biosorption for copper and zinc by selected fungi was achieved at 7.0 g of biosorbent. IR spectrum of three fungal species showed the presence of C=O groups, amine and amide N–H functional stretch.

Keywords: Biosorption, copper, electroplating effluent, fungal isolates, zinc.

WASTEWATER during electroplating activities involves poisonous heavy metals like chromium, mercury, zinc, nickel, cadmium, phosphorus and copper which build up in the food chain and cause different pathological states[1,2]. In recent years, association of different microbes has been used for wastewater treatment as bioremediators, as it was considered to have greater capability over pure and single isolates. The axenic cultures were devised to be mineralized rapidly and completely by the metabolic activity of different microorganisms[3]. The interactions between different heavy metals and microorganisms might be due to antagonistic, additive effects or synergic. These interactions might be multifaceted and distinguished which depends on the type of heavy metal ion and microbial species consortium. The collective effect of different heavy metals may be toxicity or growth stimulation in the same microbial consortium assorted from the additive effect of the single metal ions[4]. Fungi have diverse mechanisms for bioremediation of heavy metals, including metal uptake by cell-wall components, accumulation inside the cell or extracellular chelation by phytochelatins and metal lothioneins[5].

Waste samples were collected from a polluted water source near Al-Madina electroplating industry, Alipur Chattha, Pakistan, in sterilized and cleaned 1 l glass bottles with stopper. All samples were treated with 372 mg/l EDTA as the chelating agent to reduce toxicity in samples burdened with heavy metals. During examination, the samples were fresh, and were occurred immediately after return to the laboratory.

The fungi were isolated by filtration of the electroplating waste for collection of fungal propagules using 0.45 μm pore size filter paper[6]. The fungal spore suspension was prepared in 50 ml of saline solution, and 100 μl of spore suspension was inoculated in sterilized petri dish with modified culture medium consisting of aureomycin (0.35 mg/l), glucose (10 g/l), rose bengal (0.035 mg/l), $CuSO_4$ (50 mg/l), $ZnCl_2$ (50 mg/l), peptone (5 g/l) and agar (15 g/l). The incubated plates were put in an incubator at 28°C for seven days. Isolated fungal colonies were selected and inoculated on PDA slants. These slants were stored at 4°C and subculturing was repeated after every seven days interval. The total rate of occurrence was calculated as the number of species isolated from each sample divided by the total number of samples.

The fungal isolates were grown in minimal salt medium containing (g/l): $Na_2HPO_4 \cdot 2H_2O$ (7.8), KH_2PO_4 (6.8), $MgSO_4$ (0.2), $Fe(CH_3COO)_4NH_4$ (0.01), $Ca(NO_3)_2 \cdot 4H_2O$ (0.05); $NaNO_3$ (0.085) and trace element solution (1 ml/l) at pH 6.0. Incubation was carried out at 27° ± 1°C for six days in a shaker incubator at 150 rpm. Fungal biomass was splashed with sterilized water twice. Next the fungal pellets were dried at 60°C and ground with a mortar and pestle before estimation of metal removal. One gram biomass of each fungal species was used in mix culture.

To study the effect of agitation on efficiency of copper and zinc biosorption, the mixture was incubated at different agitation speeds, i.e. 100, 125, 150 and 175 rpm at 35°C for 20 min.

Adsorption of metal ions by fungal biomass was studied at pH 2, 3, 4, 5 and 6. For this, fixed biomass of 200 mg/l was added to 20 ml of effluent containing Cu and Zn at an initial concentration of 200 mg/l for 15 min.

The effect of incubation temperature on biosorption capability of fungal consortium was studied at various temperature ranges from 30°C to 40°C.

Contact time of reaction mixture is a characteristic parameter of biosorption. To study the effect of contact time, the reaction mixture was incubated for 5, 10 and 15 min.

The effect of biomass (fungal consortium) on the removal of Cu and Zn ions was studied by varying the

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biosorbent concentration from 1.0 to 7.0 g of mycelial pellets.

The effect of metal concentration on biosorption was studied by varying the concentration from 0 to 150 mg/l.

Infrared spectrum of fungal biomass was obtained using FTIR Spectrometer M 200 series (MIDAC Corp., Irvine, California, USA).

Batch biosorption experiments were performed in 250 ml Erlenmeyer flasks containing dried fungal biomass (2.0 g) in 20 ml effluent. Bioremediation was carried out at 35°C in a shaker incubator (GFL 3033) at 150 rpm for specific time intervals compared with the control (same conditions but without fungal biomass). After each test, the combination was filtered with Whatman filter paper and concentration of residual metal ions was estimated using atomic absorption spectrophotometer (M Series 08260033, Thermoelectron Corporation, UK).

The results were analysed using MINITAB (version 13.20, Minitab Inc., PA, USA) for standard deviation and regression.

Fifty different fungal species were isolated from the polluted water source. Among these *Aspergillus niger*, *Penicillium chrysogenum*, *Rhizopus oryzae* and *Mucor racemosus* have significant metal-removing capacity. These four strains were used to design fungal consortiums (C1, *P. chrysogenum*, *R. oryzae*, *M. racemosus*; C2, *A. niger*, *P. chrysogenum*, *M. racemosus*; C3, *P. chrysogenum*, *R. oryzae*, *A. niger*) and tested for biosorption efficiency (Figure 1). It was found that C3 consortium showed maximum biosorption of Cu and Zn. Previous studies reported that fungal isolates have the ability to remove poisonous metal ions from waste, e.g. *A. niger*[7,8], *Phanerochaete chrysosporium*[9], white-rot fungi[10], *M. racemosus*[11,12], *R. arrhizus*[13] and the brown-rot fungi *L. edodes*[14]. Capacity of fungi in biosorption of hazardous metals due to contain of fungi on chitin and lignin, exudation of organic acids by fungi or adsorption of metal ions to fungal cell wall[15–17].

Figures 2–4 show infrared spectra of powdered biomass, fungal pellets (heat-inactivated biomass). Characterization of functional groups on *A. niger*, *P. chrysogenum* and *R. oryzae* that are responsible for biosorption of Cu and Zn was done by using FTIR spectrometer. Analysis of *A. niger* spectrum showed peaks at 4439, 3861, 3788, 3661, 3638, 3152, 2928, 2729, 2297, 1858, 1628, 1401, 1006, 833, 704 and 663 cm^{-1}. In case of *P. chrysogenum*, the peaks ranged from 4493 to 577 cm^{-1}, while in case of *R. oryzae* the peaks were at 3843, 2954, 2624, 2298, 1858, 1628, 1402, 1007, 833, 704 and 663 cm^{-1}. IR spectrum of the three fungal species has shown that imines, amides, C–N–C bonding, hydroxyl groups stretching, C–OH stretching, oximes, and primary and secondary amines are present on their cell surface. This shows that the biosorption mechanisms on fungal biomass surface is caused by interaction between heavy metals and different fungal species. Our results are similar to earlier studies which indicated the role of functional groups on fungal cell surface and the formation of complex bond among metal ions and various functional binding groups[18,19].

The rate of biosorption increased with increase in agitation speed (Figure 5). The results also indicate that maximum biosorption of copper and zinc (90.6% and 74.9% respectively) is obtained at 150 rpm. Biosorbent removal efficiency decreased with further increase in agitation speed. Previous studies have shown that increase in agitation speed is responsible for increase in biomass, oxygen transfer and the interaction between biosorbent binding sites and metal ions[20–22].

As shown in Figure 6, maximum biosorption of Cu and Zn ions (48.5% and 0.7% respectively) is obtained at pH 4.0. The results also show that the bioremediation efficiency of fungal groups decreases with increase in pH. This increasing in biosorption efficiency due to the surface functional groups of biomass and heavy metal trapping depend directly on pH of solution[8,23,24]. Our results are similar to earlier studies which reported optimal biosorption of Zn and Cu by *Saccharomyces cerevisiae* between pH 4.0 and 5.0 (refs 25–27) and also that further increase in pH decreased solubilization of heavy metals, which makes sorption studies difficult.

Figure 7 indicates that in general, the rate of metal uptake increases with increase in temperature. Our results show an optimum temperature of 40°C for maximum biosorption copper and zinc at 50.9% and 71.2% respectively. This positive effect in biosorption efficiency might be due to increase in active sites by bond breaking at high temperatures. The temperature affects the cell wall structure and its fidelity[28,29]. The enhanced bioremediation at higher temperature is caused by the increase in binding sites on biomass for heavy metals[30].

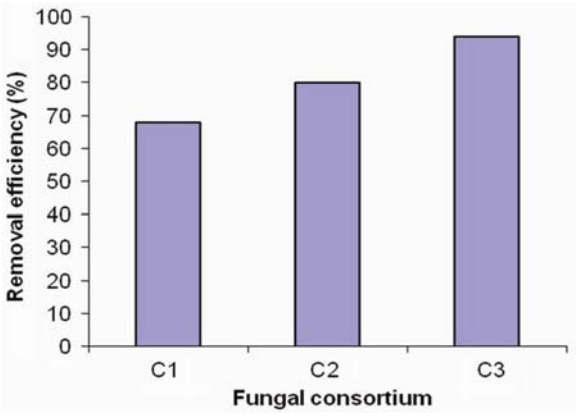

Figure 1. Removal efficiency of fungal consortiums for copper and Zinc. Removal conditions at incubation temperature 35°C, incubation time 20 min, biosorbent dose 1.0 g, pH 4.0, concentration metal of 78.0 mg/l, and agitation 150 rpm. Fungal consortiums are C1, *Penicillium chrysogenum*, *Rhizopus oryzae*, *Mucor racemosus*; C2, *Aspergillus niger*, *P. chrysogenum*, *M. racemosus*; C3, *P. chrysogenum*, *R. oryzae*, *A. niger*.

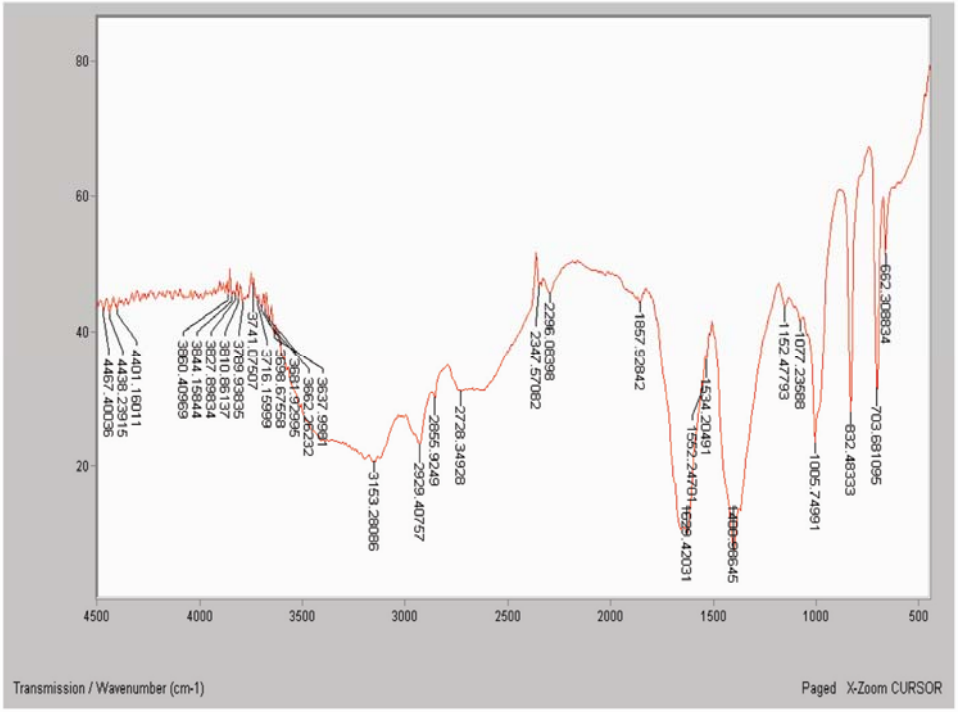

Figure 2. FTIR spectrum of *Aspergillus niger* (ASP_4).

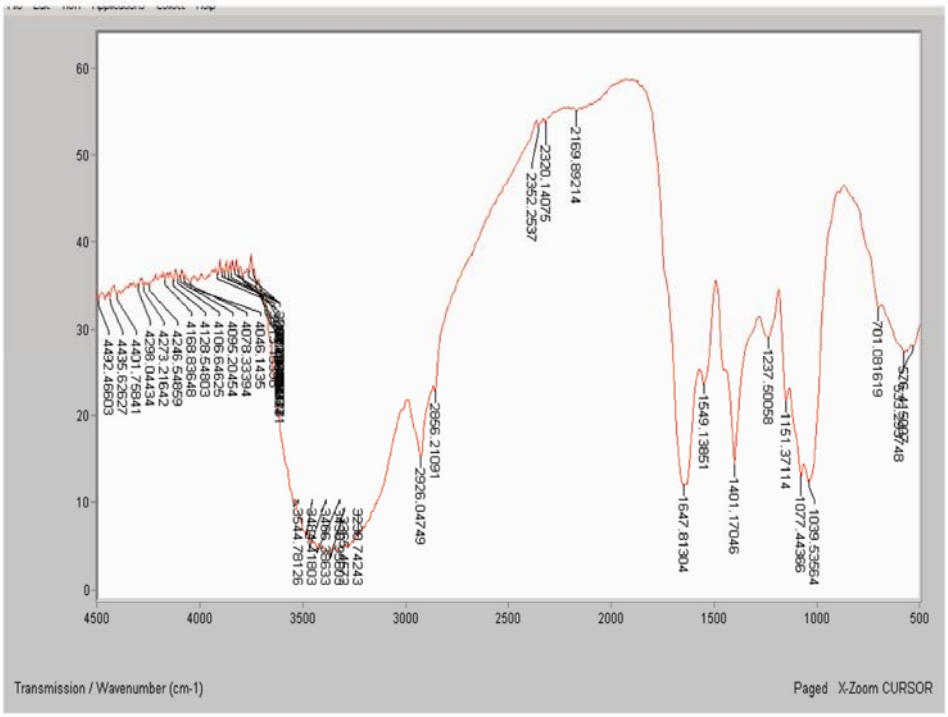

Figure 3. FTIR spectrum of *Penicillium chrysogenum* (PEN_2).

Figure 8 shows a gradual increase in biosorption rate of Cu and Zn with increase in incubation time. Optimal incubation time was 14.5 min for maximum biosorption of copper and zinc, i.e. 53.7% and 84.4% respectively. Further increase in incubation time did not positively affect biosorption (Figure 8). Our results are in agreement with previous studies which reported highest metal biosorption at incubation time of 5–15 min (refs 11, 25).

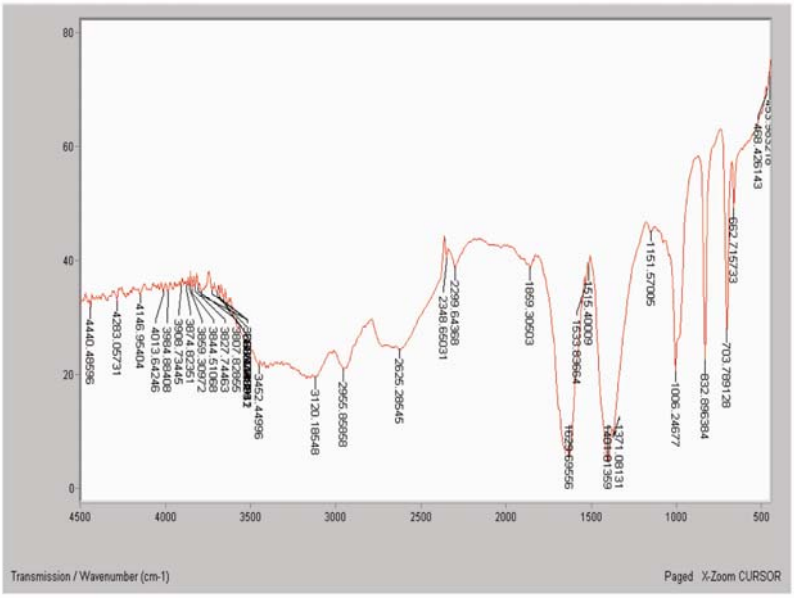

Figure 4. FTIR spectrum of *Rhizopus oryzae* (RHI_3).

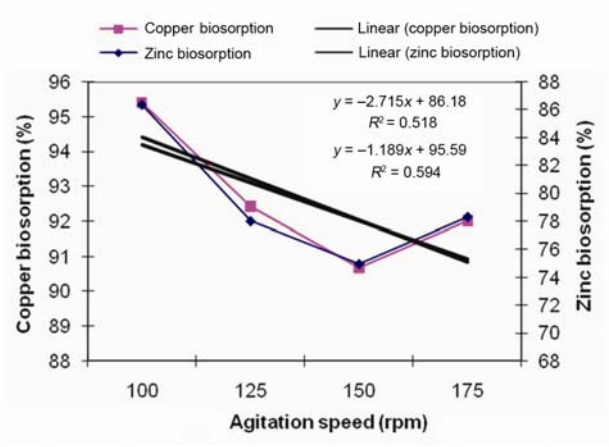

Figure 5. Effect of agitation on biosorption of metals (Cu and Zn). Concentration of metal 78.0 mg/l, pH 5.0, incubation time 15 min, temperature 35°C and biosorbent dose 2.0 g.

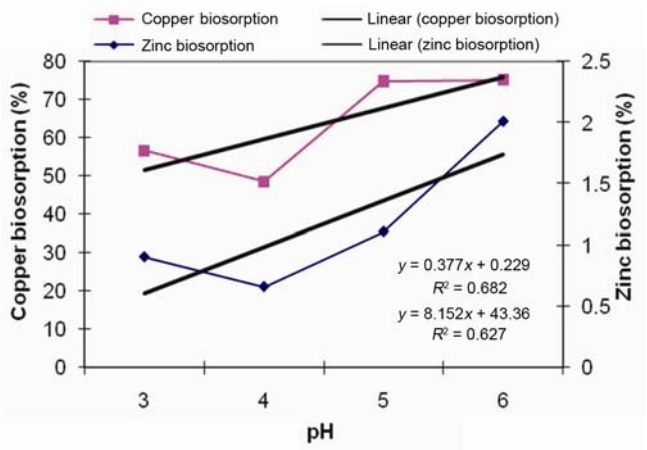

Figure 6. Effect of pH on biosorption of metals (Cu and Zn). Concentration of metal 78.0 mg/l, agitation 150 rpm, incubation time 15 min, temperature 35°C and biosorbent dose 2.0 g.

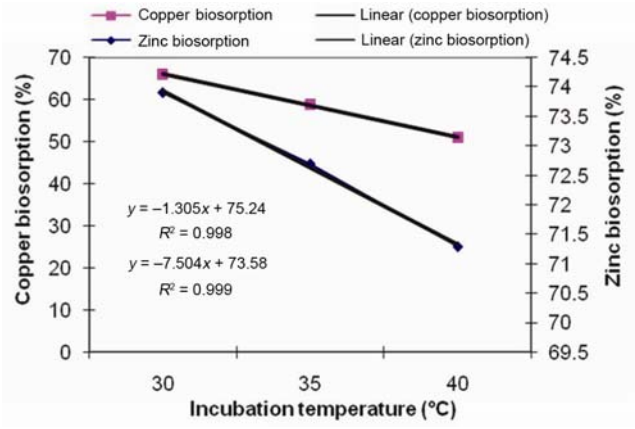

Figure 7. Effect of incubation temperature on biosorption of metals (Cu and Zn). Concentration of metal 78.0 mg/l, agitation 150 rpm, incubation time 15 min, pH 4.0 and biosorbent dose 2 g.

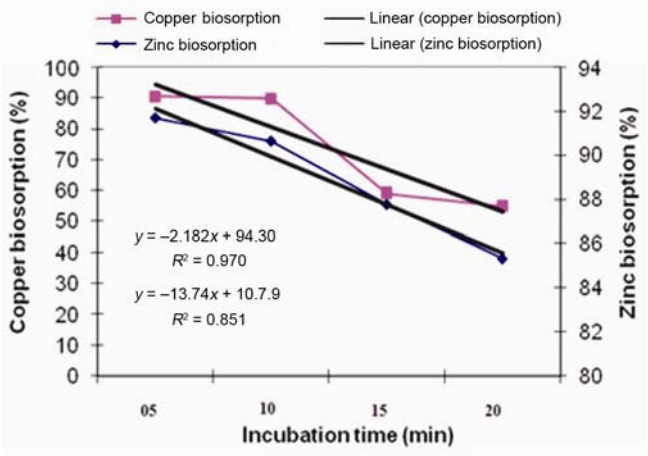

Figure 8. Effect of incubation time on biosorption using metals (Cu and Zn). Concentration of metal 78.0 mg/l, agitation 150 rpm, incubation temperature 40°C, pH 4.0 and biosorbent dose 2 g.

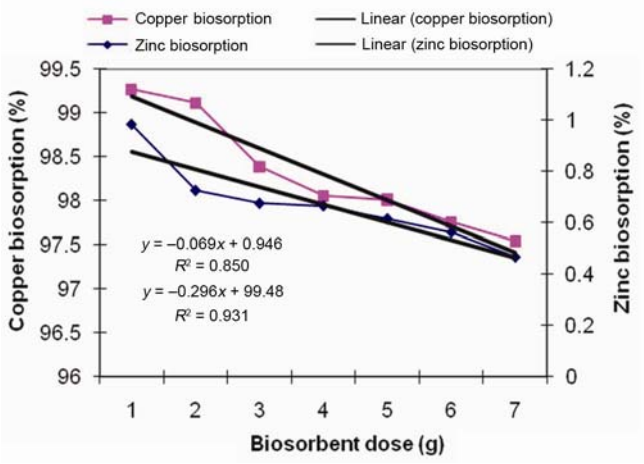

Figure 9. Effect of biomass on biosorption of metals (Cu and Zn). Concentration of metal 78.0 mg/l, agitation 150 rpm, incubation temperature 40°C, incubation time 15 min and pH 4.0.

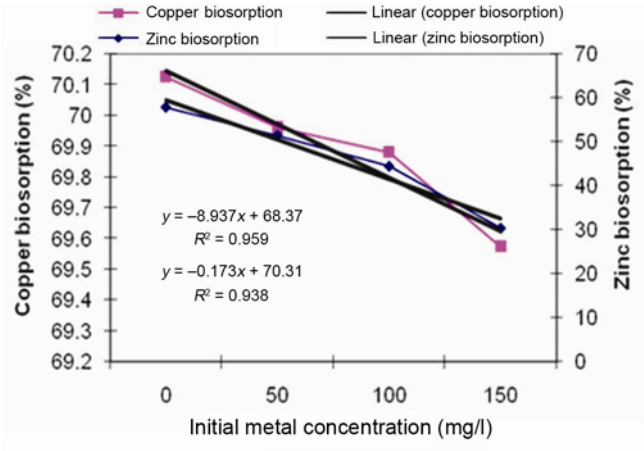

Figure 10. Effect of initial concentration of metals (Cu and Zn) on biosorption. Agitation rate 150 rpm, incubation temperature 40°C, incubation time 15 min, biosorbent dose 7 g and pH 4.0.

Figure 9 shows optimal biomass weight of 7.0 g for the maximum biosorption of copper and zinc, i.e. 98% and 0.5%. Studies[31,32] have reported that increase in fungal biomass dose generally increases the metal uptake rate due to increase in the number of binding sites. Hence increase in the biosorbent surface area, followed by slower metal deposition indicate a secondary metal-binding mechanism[33].

Figure 10 shows optimal metal concentration of 150 mg/l for maximum removal of copper and zinc, i.e. 68.5% and 31.3% respectively. Biosorption efficiency of Cu and Zn ions decreased with further increase in metal concentration. Previous studies have indicated that efficiency of metal uptake by fungi increase result in rise in electrostatic interactions, involving spots of steadily inferior for heavy metals[7,34].

Thus the fungal consortium (*P. chrysogenum*, *A. niger* and *R. oryzae*) shows great potential for removal of copper and zinc from electroplating waste. Using fungal species in bioremediation of heavy metals is one of the most common methods for their removal from electroplating effluent. The present study clearly shows that the optimization of various parameters maximizes the metal removal efficiency of the fungal consortium.

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Long-term exposure to combined treatment of elevated CO_2 and salt induces iron deficiency responses in *Porteresia coarctata*

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Plants with rising atmospheric carbon dioxide (CO_2) level in the environment may change their nutrient demands to sustain growth. The mechanisms concerning iron dynamics in plants under the interactive effect of salinity and elevated CO_2 are poorly understood. This study examines the effects of long-term as well as short-term growth at elevated CO_2 and salt on iron deficiency-associated molecular responses of *Porteresia coarctata* through analysing the transcript expression of iron deficiency-responsive genes in the leaf tissue. Plants were grown in hydroponic media at ambient or elevated atmospheric CO_2 (500 µl l^{-1}), with or without salt, and samples were analysed at three time points, on the 15th, 45th and 90th day. The semi-quantitative RT-PCR analysis showed an induced expression of iron deficiency-responsive transcription factor *PcIDEF1* and its putative targets *OsIRO2-like gene*, *OsNAAT1-like gene*, *OsNAS1-like gene*, *OsYSL2-like gene* and *PcIRT1* at elevated CO_2 with NaCl. Furthermore, a positive correlation in gene expression was observed between *PcIDEF1* and its putative targets in the 15th and 45th day samples. By contrast, in the 90th day sample, correlation in gene expression was less evident. Our findings suggest that the interactive effect of elevated CO_2 and NaCl can induce a set of molecular responses in *P. coarctata* for enhanced iron uptake and utilization, thereby reflecting an iron deficiency like stress under such conditions.

Keywords: Calcareous soil, elevated carbon dioxide, iron-responsive genes, *Porteresia coarctata*, salinity.

ATMOSPHERIC carbon dioxide (CO_2) concentration (hereafter referred to as (CO_2)) has increased from 280 µl l^{-1} in pre-industrial era to 367 µl l^{-1} at present. It is further expected to reach 550 µl l^{-1} in the coming 40–60 years[1]. As CO_2 is the main substrate for photosynthesis, its elevated level in the atmosphere has an acute impact on plant growth. The elevated CO_2 or eCO_2 increases the rate of carboxylation of ribulose 1,5-bis phosphate carboxylase/oxygenase (Rubisco) and suppresses its oxygenation particularly in C3 plants, thereby increasing the net

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