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# Heavy Metals Tolerance and Biosorption Potential of White Rot Fungi

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Potentiometeric sorption experiments were conducted for screening of cost-effective 11 strains of white rot fungi isolated from metal contaminated soils of Pakistan. In potentiometeric experiments, it was found that the uptake of the metal ions by the different strains was accompanied with proton release, indicating that the metal binding occurs *via* an ion exchange as well as by electrostatic interaction between functional groups and heavy metal ions. The minimum inhibitory concentrations (MIC) suggested that the metal toxicity was dependent on fungal isolates. All white rot fungi showed highest toxicity to Pb(II), Cr(III) and Cr(VI), whereas, Cu(II) and Zn(II) were found to be least toxic heavy metals. *Ganoderma lucidum, Agaricus bitorquis* ($J_{77}$) and *Pleutrotus sajor-caju* were more resistant to all heavy metals in comparison to other fugal isolates. The heavy metal sequestration ability of white rot fungi was also studied. Furthermore, conductometeric titrations were carried out for evaluation of ionizable functional groups. The results of the present study clearly demonstrates that different strains of white rot fungi may have a potential for use as high-value biosorbent of heavy metals and it deserves further investigations for practical applications.

**Key Words: White rot fungi, Toxicity, Conductometery, Heavy metals, Potentionmetery.**

## INTRODUCTION

One of the major environmental problems facing the world today is the contamination of water and soil by toxic heavy metals[1]. Metals can directly and indirectly damage DNA and that means an increased risk of cancer. This is known as genotoxicity. There are also possibly non-genotoxic pathways, due to irritation or immuno-toxicity. Most of the heavy metals (Pb, Co, Cr, *etc.*) have no known biological function in living organisms. Others (Cu, Zn and Ni) are thought to be essential at low concentrations, but are toxic at high levels. Fungal bioremediation (mycoremediation) has recently attracted attention of scientists to overcome pollution.

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The phenomenon of biosorption is a metabolism independent adsorption of heavy metals based on the partition process on a microbial biomass[2,3]. White-rot fungus can grow in a wide temperature range[4] and can withstand toxic levels of most organopollutants[5]. But heavy metal toxicity of white rot fungi still needed to be explored. White rot fungi is a physiological grouping of fungi that can degrade lignin and lignin like substances[6]. White rot fungi use carbon containing substances as source of energy, not the lignin. Also, the branching, filamentous mode of fungal growth allows for more efficient colonization and exploration of contaminated soil[7-9].

Potentiometery is based on the measurement of cell potentials in the absence of currents. The ability to model proton and metal adsorption onto biomass cell walls, a growing number of potentiometric studies of the protonation of cell walls and to interpret these potentiometric data have been conducted recently[10-17]. Biomass surface protonation serves two purposes: (1) to elucidate the molecular-scale mechanisms for adsorption reactions that occur between aqueous solutes and the biomass surface and (2) to enable quantitative geochemical modeling of mass transport in biomass-bearing systems. Fungi are known to tolerate and accumulate heavy metals. However, fungi may subject to toxic effect of heavy metals at elevated concentration[18]. Potential of filamentous fungi in bioremediation of heavy metal containing industrial effluents and wastewaters has been increasingly reported from different parts of the world[19]. Before the selection of fungi for removal of heavy metals from environment, it is necessary to determine, their metal tolerance ability or minimum inhibitory heavy metal concentration.

White-rot fungi are wood decomposing basidiomycetes which are capable of degrading not only lignin, but also a variety of recalcitrant environmental pollutants. Decomposition of organic pollutants by various white rot fungi has been studied widely. But the heavy metal uptake potential of white rot fungi is still needed to be explored on international science scene. Thus, the purpose of the investigation is to obtain filamentous fungi from polluted habitats for their possible exploitation in biosorption studies. The first step in selection of white rot fungi for heavy metal uptake is the determination of their metal tolerance ability. It is also obvious that most of the white-rot fungi are not able to grow into soil and therefore they are not useful for the bioremediation of contaminated soil. In the present study, we have isolated 11 white rot fungal strains *viz. Lintus edodes, Podaxis pistillaris, Pleutrotus ostreatus, Pleutrotus ostreatus* (small stem), *Pleutrotus sajor-caju, Ganoderma lucidum, Agaricus bisprous, Agaricus bitorquis* ($J_{77}$), *Agaricus bitorquis* ($A_{61}$), *Agaricus bitorquis* ($A_{65}$) and *Agaricus bitorquis* ($A_{67}$) from Pakistani metal contaminated agricultural soils receiving long-term (> 50 years) application of municipal wastewaters and industrial effluents. A long term metal exposure in the soil fungi can lead to physiological adaptation or the selection of mutants[19] and such changes may be associated with increased metal sorption capacity. In this study, we have conducted potentiometric and conductometeric titration studies of eleven white rot fungi as a function of pH. The objectives of the study are to determine the effect of solution pH on the cell wall functional groups protonation and to determine minimum inhibitory heavy metal concentration of important white rot fungi.

## EXPERIMENTAL

All the reagents used in the present study were of analytical grade and mainly purchased from Fluka Chemicals.

**Microorganism collection, identification and preparation:** Agricultural soils irrigated with municipal wastewater/industrial effluents were visited to isolate multi metal tolerant white rot fungi by the standard spread plate method in the potato dextrose agar (PDA) medium. Streptomycin sulfate was used to inhibit bacterial growth. Pure cultures of common fungi were tentatively identified at the genus level on the basis of macroscopic characteristics (colonial morphology, colour, texture, shape, diameter and appearance of colony) and microscopic characteristics (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia and presence of sterile mycelium) in the Mushroom Laboratory, Institute of Horticultural Sciences, University of Agriculture, Faisalabad, Pakistan. All white-rot fungi including *Lintus edodes, Podaxis pistillaris, Pleutrotus ostreatus, Pleutrotus ostreatus* (small stem), *Pleutrotus sajor-caju, Ganoderma lucidum, Agaricus bisprous, Agaricus bitorquis* ($J_{77}$), *Agaricus bitorquis* ($A_{61}$), *Agaricus bitorquis* ($A_{65}$) and *Agaricus bitorquis* ($A_{67}$) were cultivated in liquid medium using the shake flask method[20,21]. The growth medium consisted of (g/L); D-glucose (5.0); $KH_2PO_4$ (5.0); $MgSO_4{\cdot}7H_2O$ (0.2); $NH_4NO_3$ (2.0); $(NH_4)_2SO_4$ (4.0); peptone (2.0); trisodium citrate (2.5) and yeast extract (1.0). The pH of the medium was adjusted to 4.5 before autoclaving. The whole medium was autoclaved for 15 min. Once inoculated, flasks were incubated on an orbital shaker at 150 rpm for 7 days at 30 °C.

**Determination of minimum inhibitory concentration (MIC):** White rot fungi heavy metal tolerance was determined as the minimum inhibitory concentration (MIC) against the test fungi. In preliminary studies potato dextrose agar (PDA) and Sabouraud Dextrose Agar (SDA) mediums were selected to grow the white rot fungi. All the white rot fungi selected in the present study have shown very slow growth on SDA. Thus, PDA was mixed with an inoculum of test fungi and amended with various heavy metals concentrations to achieve the desired concentration ranging from 1 to 2000 mg/L. The plates were incubated at 30 ± 1 °C for 3 d to observe the growth of fungi. MIC was defined as the minimum concentration of the heavy metal that inhibited visible growth of test fungi[22].

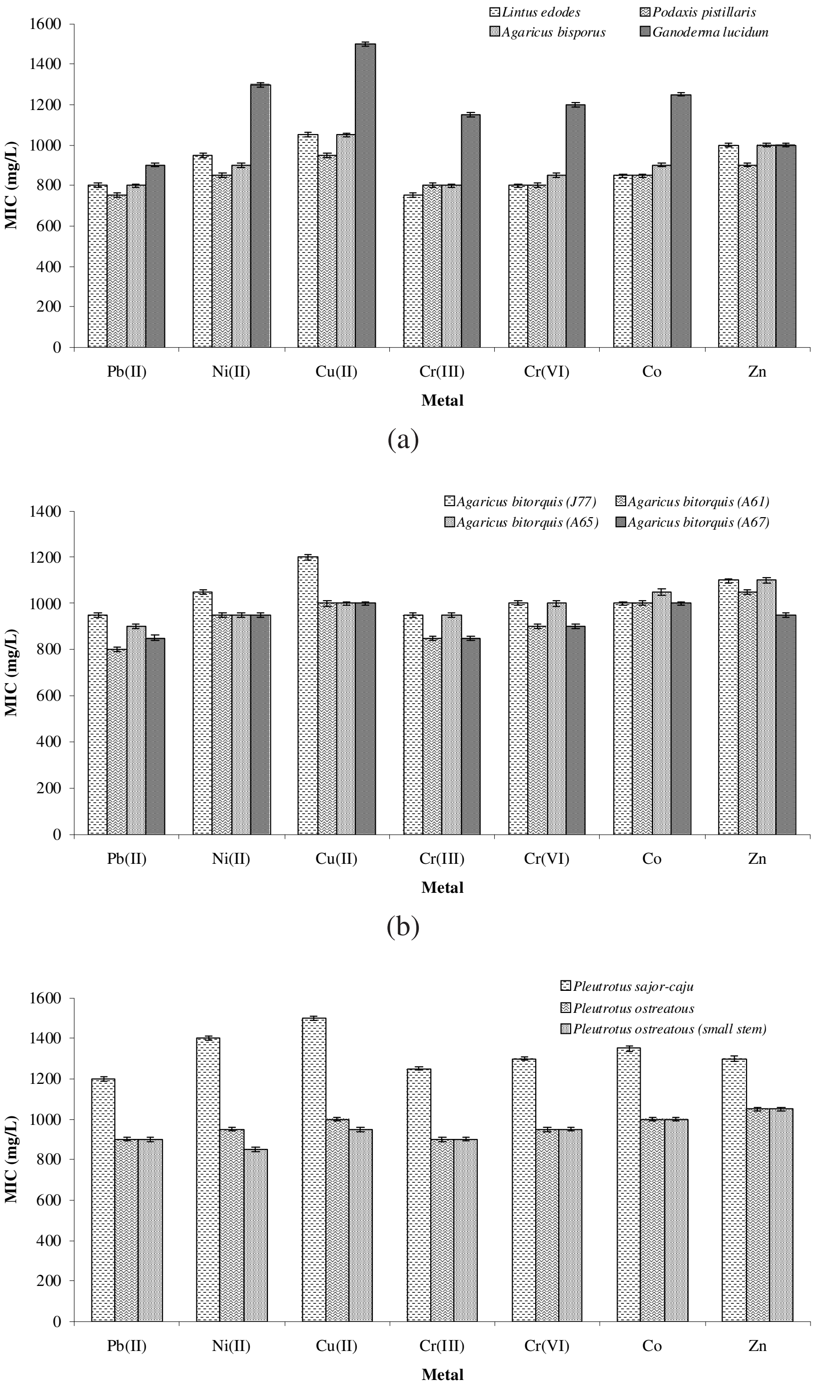
**Potentiometiometeric and conductometeric measurements:** After incubation, the biomass was harvested from the medium and washed with distilled water. The biomass was washed, centrifuged, washed, lyophilized and stored at 4 °C for biosorption tests. One gram of lyophilized biomass was protonated by adding 50 mL of 0.1 N HCl prepared in deionized distilled water. Then the suspension was placed in orbital rotary shaker at 250 rpm and 30 °C for 1 h, which was subsequently centrifuged at 6000 × g for 15 min and finally filtered using disposable Whatman filter paper of 0.45 mm pore size. The pellet was re-suspended in HCl. The previous treatment procedure was repeated three times to ensure full protonation of biomass. The protonated biomass was washed extensively with deionized distilled water. One gram of protonated lyophilized biomass suspended in 20 mL of deionized distilled water was potentiometrically and conductometerically titrated against 0.1 N NaOH as reported by Veglio *et al.*[23]. pH and conductivity measurements were calibrated against Fischer and Oakton calibration standards, respectively.

**Statistical analysis:** All experiments were performed in triplicate. The results are presented as mean ± SD.

## RESULTS AND DISCUSSION

**Determination of minimum inhibitory concentration (MIC):** The MIC values of various white rot fungi suggested that the resistance level against individual metals was dependent on the isolates (Fig. 1a-c). All the white rot fungi exhibited least metal toxicity to Cu(II) and Zn(II), suggesting that these metals play somewhat vital role in their growth on metal contaminated soils. Lead(II), Cr(III) and Cr(VI) were found to be most toxic metals to white rot fungi. Nickel(II) and Co(II) showed intermediate effect. The three isolates *Lintus edodes*, *Podaxis pistillaris* and *Agaricus bisprous* showed relatively low tolerance to all metals in comparison to other fungal isolates. Three isolates *Ganoderma lucidum, Agaricus bitorquis* ($J_{77}$) and *Pleutrotus sajor-caju* exhibited extremely high heavy metal toxicity. These fungi showing high tolerance to toxic metals may be useful in metal recovery systems. *Pleutrotus ostreatus, Pleutrotus ostreatus* (small stem), *Agaricus bitorquis* ($A_{61}$), *Agaricus bitorquis* ($A_{65}$) and *Agaricus bitorquis* ($A_{67}$) showed considerable heavy metal tolerance as exhibited by their MIC values. The variation in the metal tolerance might be due to the presence of one or more types of tolerance strategies or resistance mechanisms exhibited by different fungi. The occurrence of various other fungi species in soil polluted with heavy metals has also been reported by other workers[19,22,24].

**Biomass screening and characterization:** In order to screen suitable biosorbents which will be able to sequester the largest amounts of heavy metals from the contaminated wastewater, titration of protonated biomass was performed as preliminary test[25,26]. This preliminary biosorbent selection procedure is even more prevalent if metal uptake process is largely ion-exchange based[23]. Potentiometeric titrations was used to estimate the biomass ability to sequester heavy metal and the suitable range of pH for heavy metal adsorption. The results of the potentiometeric titrations are nearly identical for each biomass examined (Fig. 2). Alternatively the conductometeric studies were also performed. At the beginning of the conductometeric titrations, conductivity decreased sharply due to neutralization of protons from strong acid groups. The subsequent increase in solution conductivity was due to slow neutralization of weak acid groups. Solution pH affects the solubility of metal ions and concentration of the counter ions on the functional groups of the biomass cell walls[27-29]. Resultantly, potentiometeric/conductometeric titrations can

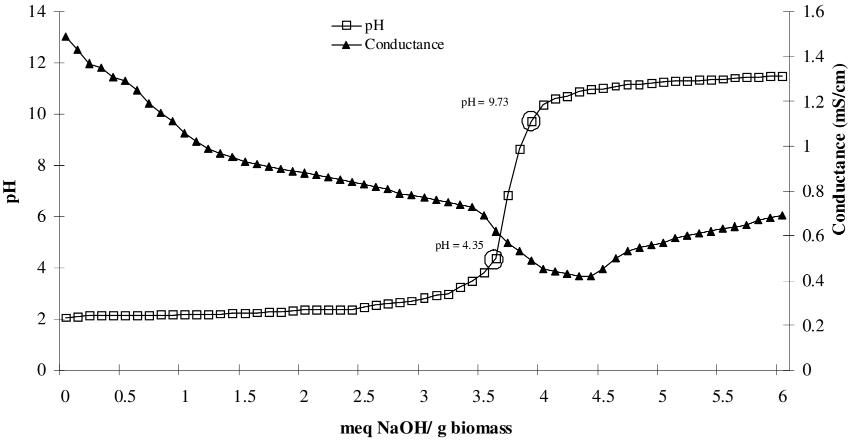


(a)

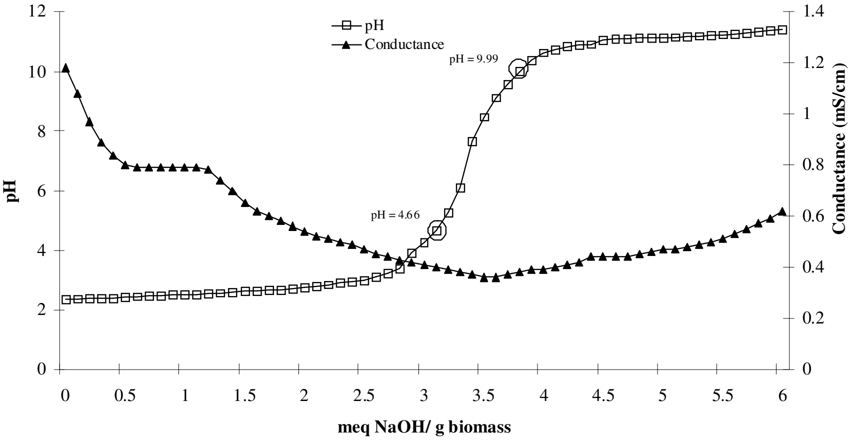
(b)

(c)

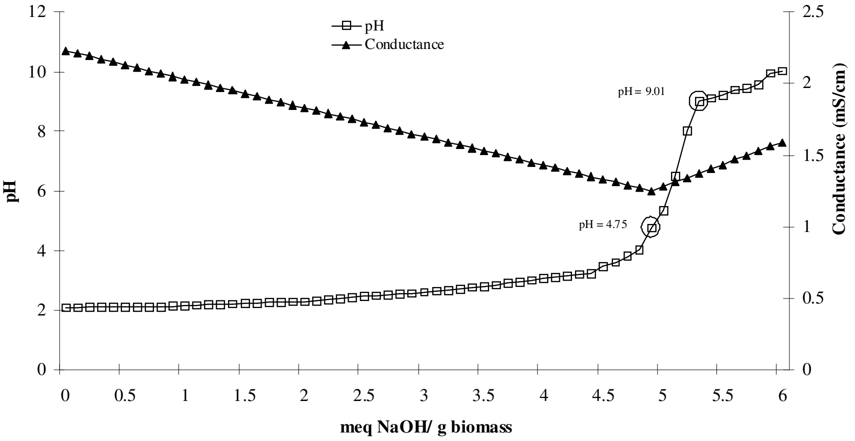
Fig. 1a-c. Minimum inhibitory concentrations (MIC) of white rot fungi



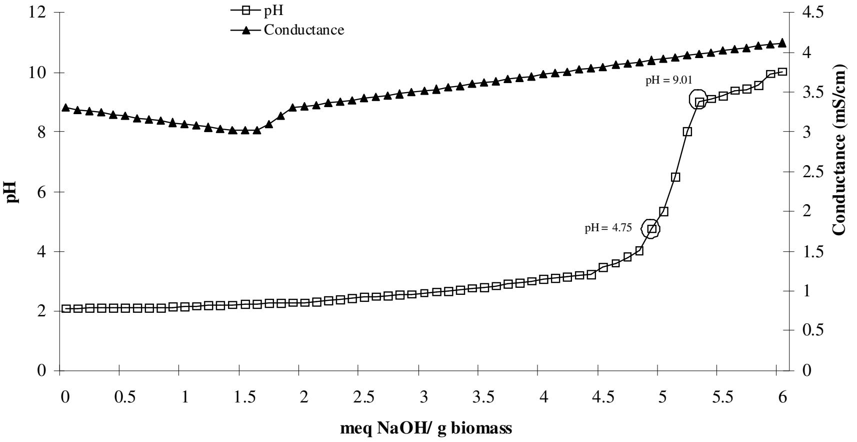
(a) *Lintus edodes*



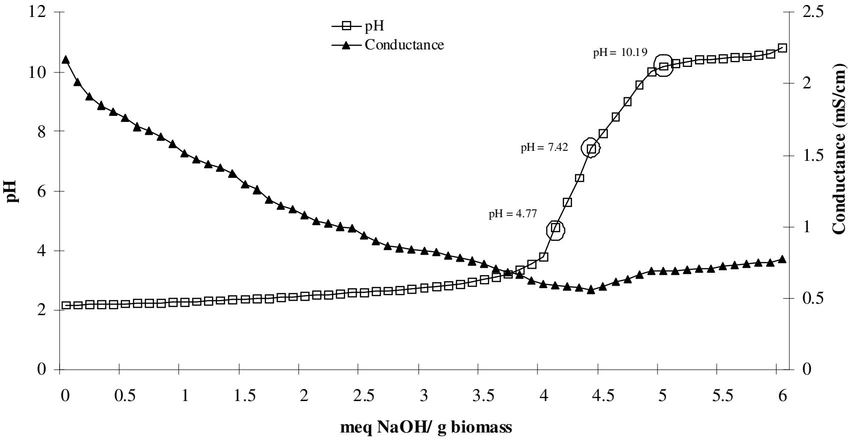
(b) *Agaricus bisprous*



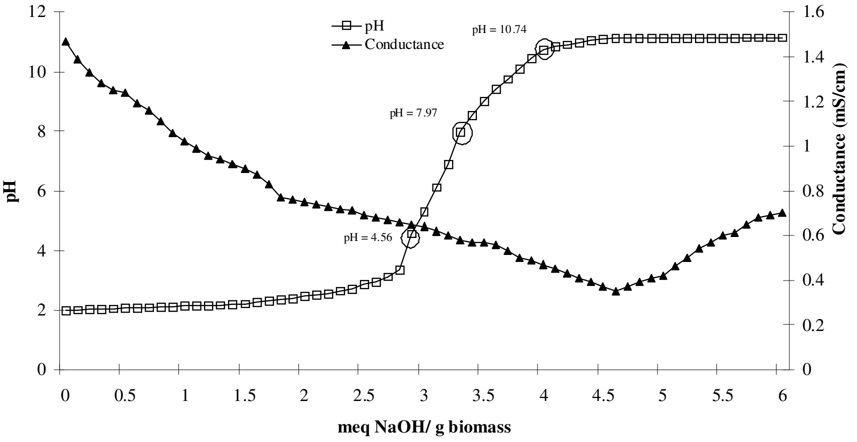
(c) *Podaxis pistillaris*



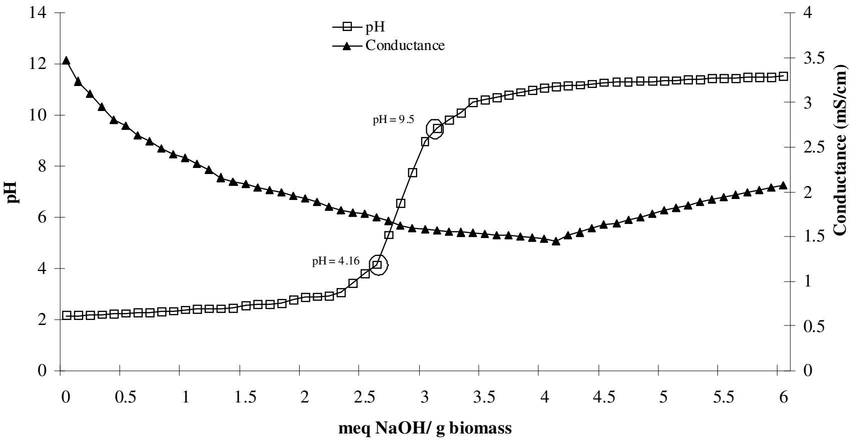
(d) *Ganoderma lucidum*



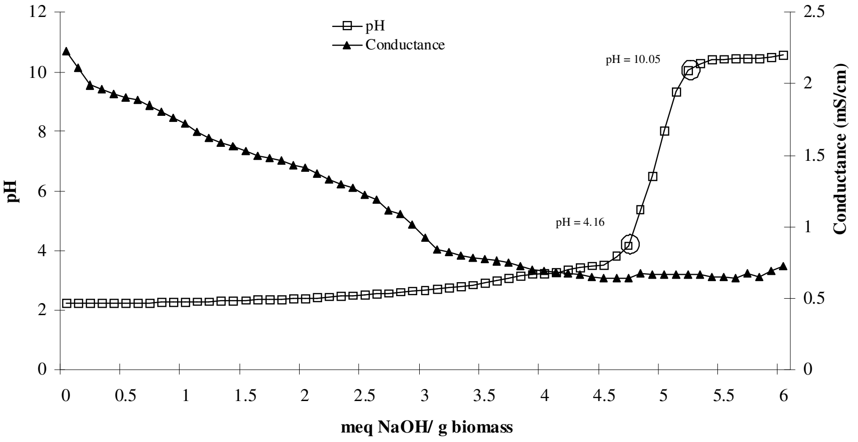
(e) *Agaricus bitorquis* ($J_{77}$)



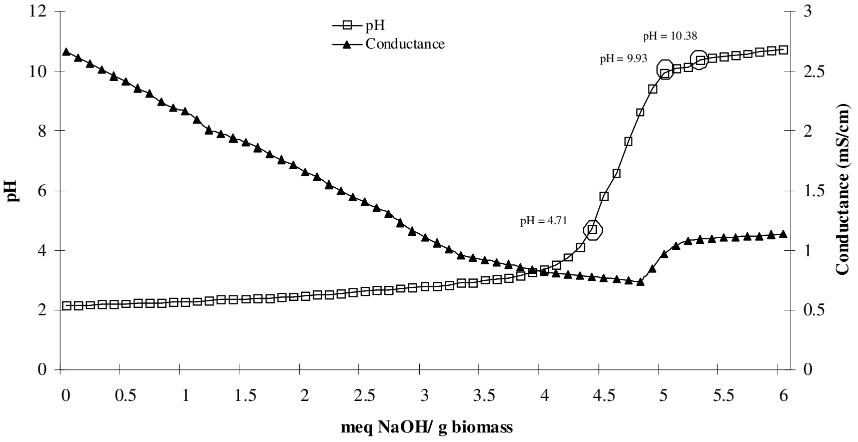
(f) *Agaricus bitorquis* ($A_{65}$)



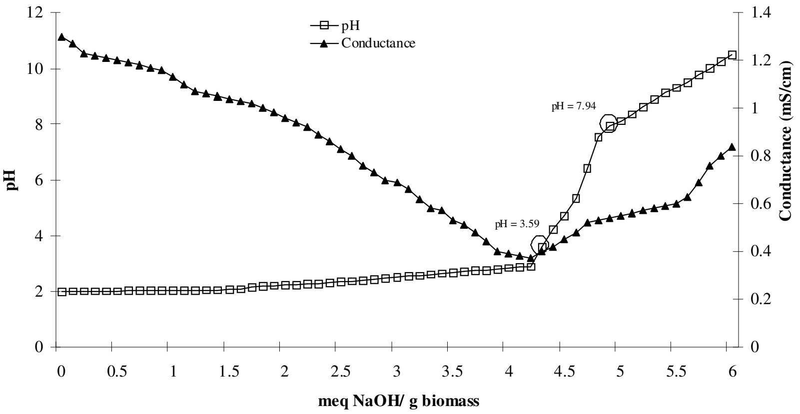
(g) *Agaricus bitorquis* ($A_{61}$)



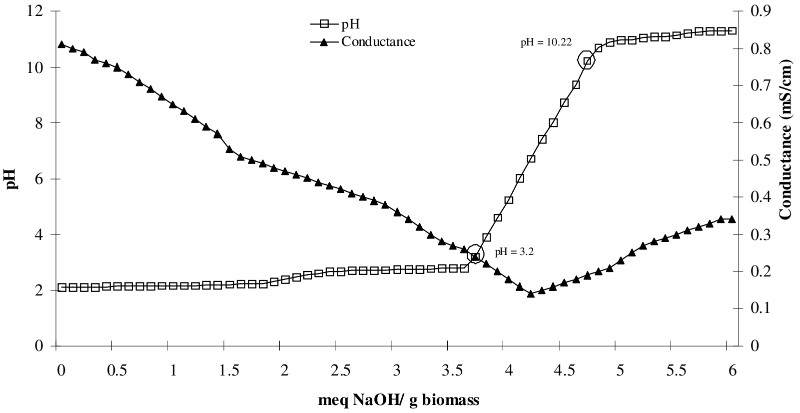
(h) *Agaricus bitorquis* ($A_{67}$)



(i) *Pleutrotus sajor-caju*



(j) *Pleutrotus ostreatus*



(k) *Pleutrotus ostreatus* (small stem)

Fig. 2a-k. Potentiometeric and conductometeric titration of white rot fungi

provide rough estimation of biosorptive potential of any biomass. These are particularly useful for the evaluation of ionizable groups such as carboxyl, aldehydic, ketonic, alcoholic and amino groups. It is proposed for the first time that the relative heavy metal (RHM) uptake capacity of various functional groups present on the biomass surface after carefully understanding the basic principles of potentiometery and evaluating the experimental results (Table-1).

The number of equivalents of weak acid present can be easily calculated by eqn. 1:

Weak acid (mmol/g) = (pH before 1st equivalence point - pH after
1st equivalence point) - Strong base meq used for titration (1)

The number of equivalents of strong acid can be calculated by difference method (eqn. 2):

Strong acid (meq) = Weak acid (meq) -
Strong base meq used for titration (eq. 2)

TABLE-1
PROPOSED RELATIVE HEAVY METAL (RHM) UPTAKE CAPACITIES OF VARIOUS FUNCTIONAL GROUPS PRESENT ON BIOMASS CELL SURFACE

| Fungi | Strong acid groups (mmol/g) | Weak acid groups (mmol/g) | Aldehydic, ketonic and alcoholic (mmol/g) | Amino and other strong base groups (mmol/g) |
|---|---|---|---|---|
| *Lintus edodes* | 0.30 | 0.79 | 0.74 | 0.50 |
| *Agaricus bisprous* | 0.40 | 1.49 | 1.12 | 0.50 |
| *Podaxis pistillaris* | 0.20 | 1.13 | 0.1 | 0.1 |
| *Ganoderma ilucidum* | 0.40 | 1.35 | 0.02 | 0.40 |
| *Agaricus bitorquis* ($J_{77}$) | 0.10 | 1.76 | 0.20 | 0.11 |
| *Agaricus bitorquis* ($A_{65}$) | 0.40 | 1.29 | 0.20 | 0.40 |
| *Agaricus bitorquis* ($A_{61}$) | 0.40 | 0.85 | 0.40 | 1.12 |
| *Agaricus bitorquis* ($A_{67}$) | 0.50 | 0.31 | 0.08 | 0.30 |
| *Pleutrotus sajor-caju* | 0.60 | 1.05 | 0.12 | 0.40 |
| *Pleutrotus ostreatus* (small stem) | 0.40 | 0.04 | 0.38 | 0.50 |

The number of equivalents of aldehydic, ketonic and alcoholic groups can be calculated, if the equivalent point lies above pH 7. Following eqn. 3 can be useful for this purpose.

$$\text{Aldehydic, ketonic and alcoholic (mmol/g)} = (\text{pH before equivalence point} - \text{pH after equivalence point}) - \text{Strong base meq used for titration} \quad (3)$$

The heavy metal uptake capacity of amino and other strong base groups can be calculated using eqn. 4. Their equivalence point is above pH 10.

$$\text{Strong base groups (mmol/g)} = \text{Aldehydic, ketonic and alcoholic (meq)} - \text{Strong base meq used for titration} \quad (4)$$

The relative heavy metal uptake capacity of various functional groups by above proposed by Asif and Bhatti equations, was calculated for white rot fungi included in the present study and tabulated in the Table-1.

## Conclusion

Our preliminary findings indicated that *Ganoderma lucidum, Agaricus bitorquis* ($J_{77}$), *Pleutrotus sajor-caju* isolated from Pakistani soils contaminated with heavy metals have high metal tolerence and could be exploited for mycoremediation of heavy metals from the polluted environment. The results also indicate direct relationship between level of metal resistance and bioremediation capacity in white rot fungi. Further investigations should be focused on *Ganoderma lucidum, Agaricus bitorquis* ($J_{77}$), *Pleutrotus sajor-caju* to optimize the conditions for metal removal from multimetal aqueous solutions and diluted wastewaters for large scale operation. The Asif and Bhatti equations can be useful for evaluating sorption capacity of different functional groups using potentiometery.

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