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ORIGINAL ARTICLE

Sorption of lead and copper from an aqueous phase system by marine-derived *Aspergillus* species

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Abstract A total of 47 cultures of *Aspergillus* representing 13 species were screened for their ability to tolerate 7.5 mM Pb^{2+} and 2 mM Cu^{2+}, all of which were positive, with growth of 31 of the cultures being enhanced by low concentrations of lead. The isolates of *Aspergillus versicolor, A. niger and A. flavus* were tolerant to concentrations as high as 10 - 12.5 mM Pb^{2+} and 3 – 4 mM Cu^{2+}. Selected cultures displayed a good sorption capacity of 32 - 41 mg Pb^{2+} and 3.5 - 6.5 mg Cu^{2+} g^{-1} dry weight of mycelia, which was improved by alkali pretreatment of the biomass and negatively affected by mild dry heat treatment. The sequestration of the metal occurred mainly by sorption to the cell-surface with very little intracellular uptake. FTIR analysis indicated the involvement of hydroxyl, amino, and carbonyl groups in Pb^{2+} and Cu^{2+} biosorption by fungal biomass of the different species of *Aspergillus*.

Keywords *Aspergillus* · Heavy metals · Biosorption · Cell wall · Functional groups

Introduction

Metal pollution has been drastically increasing over the years as a result of technological and socio-economic advancement. Through the process of bioaccumulation, the metal ions concentrate and exceed the normal limits in the food chain as well as the environment, leading to serious ecological and medical problems.

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Lead pollution results from many industrial applications such as storage battery manufacturing, printing, pigments, fuels, photographic materials and explosive manufacturing (Dursun et al. 2003). Lead toxicity causes anemia, encephalopathy, hepatitis and nephritic syndrome (Waihung et al. 1999). Copper contamination comes from industrial effluents and seepage, pesticides added to soil, from old copper water pipes and corrosive water that comes in contact with pipe fittings or joints (Rehman et al. 2008); toxic levels inhibit macromolecules synthesis and other enzymatic reactions (Company et al. 2004).

Known conventional techniques, which are used in the removal of dissolved heavy metals are inefficient, expensive and not eco-friendly (Tsekova et al. 2010; Vinopal et al. 2007). Many fungi can survive and grow in high concentrations of toxic metals (Gadd 1993); these features have contributed to an alternate and more viable process for the removal of heavy metals through biosorption (Iskandar et al. 2011), through the metal-sequestering properties of the fungal cell (Wang and Chen 2006). This technology can be used to decrease the concentration of heavy metal ions in solution from ppm to ppb level.

Fungi as sorbents have greater potential for remediation by virtue of their survival in high concentrations of toxic metal, greater biomass production and extensive hyphal reach in the soil (Leitão 2009). Dried or chemically pretreated biomass is a preferred alternative to the use of living cells for the removal of heavy metal ions from wastewater in order to avoid the problems of metal toxicity on microbial growth, or energy requirement for active intracellular transport (Yan and Viraraghavan 2000; Baik et al. 2002).

Microbial biomass provides a metal sink, through sorption by the cell wall, pigment and extracellular polysaccharide, intracellular accumulation, or precipitation of metal compounds in and/or around cells, hyphae or other

structures (Macek and Mackova 2011). Functional groups such as carboxylate, hydroxyl, sulfate, phosphate and amino groups in the cell surface of the biomass play an important role in the biosorption of metal ions (Akhtar et al. 1996; Kapoor and Viraraghavan 1995).

Various researchers have investigated the use of *Aspergillus* spp. as a sorbent for removal of metal ions: *A. niger* has been shown to effectively remove uranium, lead, cadmium and copper ions (Yakubu and Dudeney 1986; Kapoor et al. 1999); Huang and Huang (1996) investigated the use of *A. oryzae* to remove cadmium and copper ions from aqueous solution and a strain of *A. terreus* has been shown to take up chromium, nickel, lead and iron from metallurgical effluents (Dias et al. 2002; Sun et al. 2010). In the same way, Rao et al. (2005), and Wang et al. (2010) reported promising biosorption of Cd^{2+}, Co^{2+}, Cu^{2+} Ni^{2+} and U^{6+} by *A. fumigatus*.

The present work is a comparative study of different *Aspergillus* species with respect to their capacity for heavy metal tolerance and removal from solution, and the mechanism of metal sequestration with regard to cell-surface sorption and intracellular uptake, and the cell wall functional groups involved in metal sorption as determined by FTIR analysis.

Materials and methods

Cultures

A total of 47 *Aspergillus* cultures, previously isolated from different ecosystems in Goa, India:- Estuary of Mandovi (EM): water - top (wt) and bottom (wb) and sediment (s); Mangroves (M) and Salterns (S) of Panjim (P), Ribander (R) and Santa Cruz (C): water (w) and sediment (s) were used in this study. The Mandovi estuary, situated on the West Coast of the Indian Peninsula, serves as a waterway for ferries as well as for barges transporting iron ore from the mining areas hinterland, to the port; it also tends to be subjected to illegal dumping of waste. The estuary is lined with mangroves, with salterns being constructed alongside the mangroves in some areas close to the mouth of the estuary. The salinity of the estuarine waters was around 34 ‰ and that of the sediment was 10 ‰; the mangroves had a salinity of about 30 ‰ and that of the saltern brine was 280 ‰. The cultures were maintained on Czapek Dox Agar containing (l^{-1}): sucrose (30 g), $NaNO_3$ (2 g), K_2HPO_4 (1 g), KCl (0.5 g), $MgSO_4{\cdot}7H_2O$ (0.5 g), $FeSO_4.7H_2O$ (0.01 g) and agar (15 g), amended with 2 % NaCl, and designated as S-CzA. The cultures were identified to the species level according to the key of Raper and Fennel (1965) on the basis of colony characteristics of color, texture, diameter and appearance, and microscopic characteristics: septation in mycelium, presence of specific reproductive structure, shape and structure of conidia.

The salt tolerance of selected isolates of these marine fungi was examined by growing them on CzA supplemented with solar salt from 0-15 %. Tolerance to increased levels of salt would serve as an additional advantage for potential use of the isolates in bioremediation of saline systems.

Metal tolerance by aspergilli

Spore suspensions of the cultures were prepared by suspending spores from a 2 % S-CzA into 2 % NaCl solution containing 0.05 % Tween 80; the spore concentration was obtained using a haemocytometer. The cultures were initially screened for tolerance to Pb^{2+} and Cu^{2+} ions by spot inoculation of the spore suspension on to S-CzA plates containing 7.5 mM Pb^{2+} as $Pb(NO_3)_2$ and 2 mM Cu^{2+} as $CuSO_4{\cdot}5H_2O$, incubated at 30 °C up to 5 days; the growth was recorded in terms of colony diameter. Those cultures that exhibited high tolerance to heavy metals, were examined for their maximum tolerance concentration (MTC) of Pb^{2+} and Cu^{2+}. Spore suspensions, (10^3 spores ml^{-1}) were spot-inoculated in triplicate on S-CzA containing 0, 5, 7.5, 10, 12.5 and 15 mM Pb^{2+} as $Pb(NO_3)_2$ or 0, 1, 2, 3, 4 and 5 mM of Cu^{2+} as $CuSO_4{\cdot}5H_2O$ and incubated at 30 °C; growth was recorded in terms of colony diameter after 4 days and examined visually for changes in growth pattern, sporulation and pigment production up to 7 days, compared against the control of cultures grown in the absence of the heavy metal. MTC was defined as the highest concentration of heavy metal that the organism can tolerate.

Metal biosorption

Selected cultures from different econiches, were studied for their sorption capacity of heavy metals. Spore suspension (10^6 spores) of a freshly grown agar culture was inoculated in 100 ml of S-CzB and incubated for 3 days at 30 °C, 150 rpm; the biomass was then harvested by filtering through double layered muslin cloth and washed with deionized water. Mycelial biomass, 1 g wet weight, was resuspended in 20 ml of 1 mM each of Pb^{2+} or of Cu^{2+} as $Pb(NO_3)_2$ and $CuSO_4{\cdot}5H_2O$. Control flasks containing only metal solution without biomass, and, biomass in suspension without metal, were also maintained. The flasks were placed on a rotary shaker for 1 h at 30 °C at 150 rpm. The suspension was then centrifuged at 5009 *g* for 20 min to separate the biomass and pH of the supernatant was measured with Eutech pH meter. The supernatants and metal control were digested with concentrated HNO_3 for 10 min and then made to original volume with 1 % HCl. The heavy metal concentration was analyzed by atomic absorption

spectrophotometry (AAS) using Shimadzu AA-6300. All the experiments were carried out in triplicate.

The amount of metal sorbed by the fungal biomass was calculated from the differences between the initial metal concentration and the residual concentration after sorption, using the following equation $Q = V(C)_I - C_f)/S$ mg l^{-1} (Volesky and Holan 1995; Yan and Viraraghavan 2003) where Q = mg of metal ion sorbed g^{-1} dry weight biomass; C_I = initial metal concentration mg l^{-1}; C_f = final metal ion concentration mg l^{-1}; S = sorbent (g) of biomass in the reaction mixture, V = volume (L) of the reaction mixture.

Metal sorption by treated biomass

Four species of *Aspergillus* that showed high metal sorption, were selected and grown in CzB as above; the harvested biomass was subjected to dry heat treatment at 60 °C for 12 h, or boiling in solution of 5 % sodium hydroxide for 15 min followed by washing with deionized water until the pH of the solution was neutral. Both untreated and pretreated biomass of the cultures were examined for their capacity to sorb Pb^{2+} and Cu^{2+} metal from solution, as described above.

Cell-bound and intracellular metal uptake

The selected isolates were also examined for quantization of cell bound and intracellular uptake of metals. Cultures grown in S-CzB were filtered, washed and incubated in Pb^{2+} and Cu^{2+} metal solutions for 1 h, as detailed above. The residual heavy metal ions in solution were estimated to obtain the total metal sorbed, as given above. The cell-bound metal was eluted by washing the biomass with 0.1 N HCl at 100 rpm for 10 -15 min. The supernatant and washings were collected and analysed for metal content by AAS. The intracellular metal uptake was determined by digesting the washed biomass with perchloric acid, 5 ml, at 100 °C for 5 min, followed by further digestion with addition of 5 ml conc. HNO_3 till a clear solution was obtained (Ahuja et al. 2001); the digests were then made up to 10 ml in a volumetric flask with de-ionized water and subjected to AAS.

Determination of functional groups involved in metal sorption

The functional chemical groups present on the cell walls of fungal biomass that are responsible for heavy metal biosorption was analyzed by Fourier Transform Infrared (FTIR) spectroscopy. The biomass of the selected cultures before and after exposure to heavy metals, were dried at 60 °C for 12 h; finely ground biomass was encapsulated in KBr (1:10, w/w) to prepare translucent sample disks and analyzed using IR Prestige-21 FTIR- Shimadzu).

Results and discussion

Identification of species

The 47 cultures comprised of 16 species, as shown in Table 1. The *Aspergillus* cultures were identified as *A. versicolor* (11 isolates), *A. niger* (10), *A. nidulans* (5), *A. fumigatus* (4), *A. flavus* (3), *A. sydowii* (2) *A. subsessilis* (2), *A. ochraceus* (2), *A. terreus* (2), *A. oryzae* (2) and *A. candidus*. Four cultures showed the teleomorphic stage, producing sexual spores and belonged to the genera *Eurotium* (3) and *Emericella* (1).

Maximum tolerance concentration

The preliminary screening of the 47 cultures for heavy metal tolerance indicated that all of them were tolerant to 7.5 mM Pb^{2+} and to 2 mM Cu^{2+}. Twenty-two of the cultures that

Table 1 Identification of cultures

Species	Culture number
Anamorph	
A. versicolor	EM2wt41, EM2s58, EM3s110, EM3s115, EM4w119 EM7s172, MCs 220, MCs224, SRw 236, SRs 242
A. sydowii	EM5wb131, SRs246
A. niger	MRw33, MRw34, EM2wt47 EM2s111, EM4wt96 EM4s113, EM7s164, EM7s179, EM8wt175, EM9wb197
A. nidulans	MRw22, MRw31, EM2wt120, EM4wb122, SP27
A. subsessilis	EM4ws127, SRw127
A. flavus	MRw24, EM6wt140, SP22
A. oryzae	MRw19, MRw27
A. fumigatus	EM2s109, EM2s112, EM4wt117, EM4wb124
A. terreus	EM3s116, EM6s154
A. ochraceus	MRw44, MRw213
A. candidus	SRs248
Teleomorph	
Eurotium amstelodami	MRw 210
E. chevalieri	SRs 245
E. repens	MCs 218
Emericella nidulans var. *nidulans*	MRw 29

EM: Mandovi estuary, wt: water-top, wb: water-bottom, s: sediment; M: Mangroves, S: Salterns of Panjim (P), Ribander (R) and Santa Cruz (C): water (w) and sediment (s)

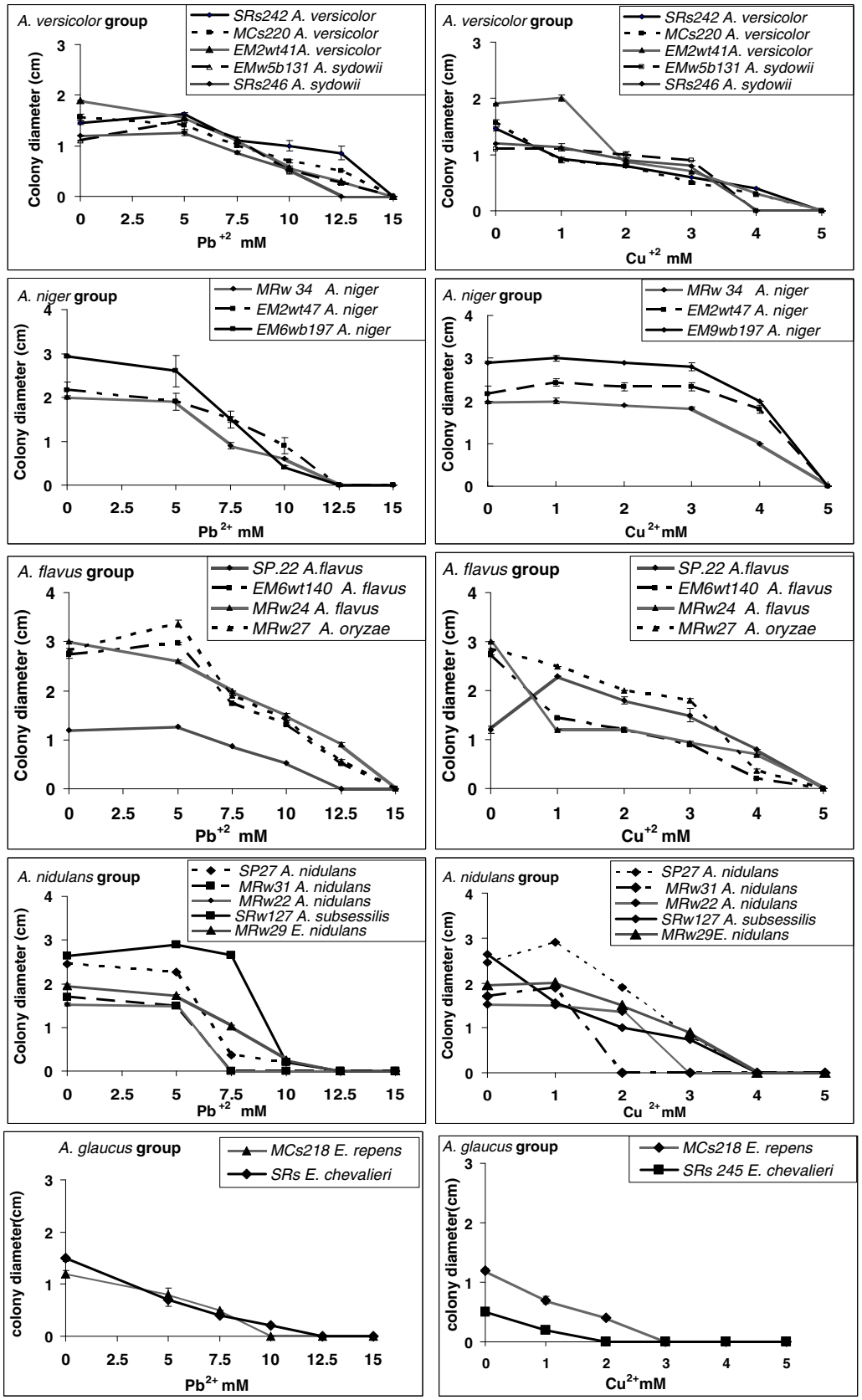

Fig. 1 Tolerance of lead and copper by cultures of *Aspergillus* species

exhibited a good growth, were examined for their tolerance to the heavy metals (Fig. 1); species of *A. fumigatus* and *A. candidus* showed lower tolerance to the heavy metals and were not selected. The lower tolerance level of *A. fumigatus* to the metals corroborates earlier studies (Iskandar et al. 2011). The growth of the cultures in presence of different concentrations of the heavy metals as compared to that on media without metal, was either slower, similar, or enhanced. Most of the fungi had MTC values ranging between 7.5-12.5 mM for Pb^{2+} and 3-4 mM for Cu^{2+}; however at the MTC, the growth was sparse and without sporulation.

Cultures belonging to the groups of *A. versicolor, A. niger* and *A. flavus* were the most tolerant to lead and copper concentrations with MTC at 12.5 mM Pb^{2+} and 4 mM Cu^{2+}, with the growth of some being enhanced in presence of low concentrations of lead or of copper. Lead ions appear to be less toxic in comparison with copper, on the growth of the *Aspergillus* species; this has also been observed in isolates of *Aspergillus, Penicillium*, *Fusarium,* as well as *Corollospora lacera* and *Monodictys pelagica* (Kowshik and Nazareth 2000; Dursun et al. 2003; Sen et al. 2005; Taboski et al. 2005; Al-Kadeeb 2007; Nazareth and Marbaniang 2008; Ezzouhri et al. 2009).

The tolerance to lead or to copper was comparable to values reported for other species of *Aspergillus* and *Penicillium* (Ezzouhri et al. 2009), *Fusarium solani* (Kowshik and Nazareth 2000) and marine *Penicillium* species (Marbaniang and Nazareth 2007); however, it was seen to be less than that reported for *A. niger* strains (Ezzouhri et al. 2009; Iskandar et al. 2011) and a deep-sea isolate of *Penicillium* (Sun and Shao 2007). The stimulation of growth at lower concentrations of Pb^{2+} and of Cu^{2+} has also been reported by Al-kadeeb (2007). The *Aspergillus* anamorph had a higher tolerance to heavy metals than its teleomorphic state of *Eurotium* and *Emericella* groups.

The results obtained thus affirmed that the response of the cultures to heavy metals varied according to the group and the species.

Metal biosorption

Sixteen cultures were selected based on the highest MTC values obtained, the difference in species and groups, and the econiche from where they were isolated. The biomass of most of the isolates could sorb Pb^{2+} with a Q value of 30-40 mg g^{-1} dry weight biomass and 3-7 mg Cu^{2+} g^{-1} dry weight biomass (Fig. 2A), with the development of a pale grey colour in presence of lead ions and a light blue in presence of copper; the supernatent after metal sorption was found to have a pH of 6. These values for lead sorption were higher than that obtained in some studies, namely 22 mg g^{-1} by *Aspergillus versicolor* (Cabuk et al. 2005) and 6 mg g^{-1} and by marine fungus *Monodictys pelagica* (Taboski et al. 2005), but less than that reported for *A. niger* strain and by the marine fungus *Corollospora lacera* (Taboski et al. 2005) and alga *Ecklonia maxima* (Feng and Aldrich 2004; Iskandar et al. 2011). Similar observations were obtained for copper sorption of 3.65 mg g^{-1} by an isolate of *A. niger* (Price et al. 2001), which differed from that of 20.91 mg g^{-1} by the *A. niger* strain as reported earlier (Iskandar et al. 2011). It was observed however, that *Eurotium* cultures, the perfect state of *A. glaucus* group, showed comparatively poor sorption of the heavy metals.

These differences in metal uptake between the species could be due to inter-species variations in the chemical composition of cell walls (Gadd 2009), the interaction of metal ions in solution with the cell surface or extra cellular polysaccharide, proteins and chitins playing a major role in biosorption as a mechanism of metal tolerance (Zafar et al. 2007).

The development of colour by the biomass when challenged with metal may be due to sequestration of Pb^{2+} and Cu^{2+} either by passive sorption and accumulation of the metal on the cell wall or by active uptake of Pb^{2+} and Cu^{2+} intracellularly, which thus imparts a colouration to the mycelia. This phenomenon has also been shown in *C. lacera* (Taboski et al. 2005) and *Trichoderma viride* (Anand et al. 2006) grown in presence of lead and copper respectively; a similar response by *Trichoderma atroviride* mycelia turning milky white in presence of zinc has been reported (Yazdani et al. 2010).

It was seen that the capacity of sorption varied amongst the different species, with an intra-species difference in the sorptive capacities with respect to the location or econiche from which they were isolated. Thus, the species of *Aspergillus versicolor* and *A. sydowii* under the *A. versicolor* group, isolated from Mandovi estuary (at a point fairly close to the mouth) had greater sorption capacity for lead than isolates obtained from salterns. Similarly, *A. niger* from mangroves situated fairly close to the mouth of the Mandovi estuary, sorbed more lead than the isolate obtained from the Mandovi estuary at a point away from the estuary mouth; sorption by cultures from mangroves and the estuary, all located fairly close to the mouth, was similar. Microorganisms when exposed to heavy metals are known to develop higher tolerance levels (Wood and Wang 1983). The high sorptive capacity, together with the high levels of metal tolerance by the cultures in this study, would therefore indicate that there is a fair amount of metal pollution towards the mouth of the estuary.

The results indicate the potential of the aspergilli for metal bioremediation, corroborating earlier reports on the efficiency of *Aspergillus* species: *Aspergillus niger, A. flavus* and *A. foetidus,* in biosorption of several heavy metals (Prasenjit and Sumathi 2005; Aung and Ting 2005; Akar and Tunali 2006; Santhiya and Ting 2006; Dacera and Babel 2008) and their removal from wastewater (Price et al. 2001).

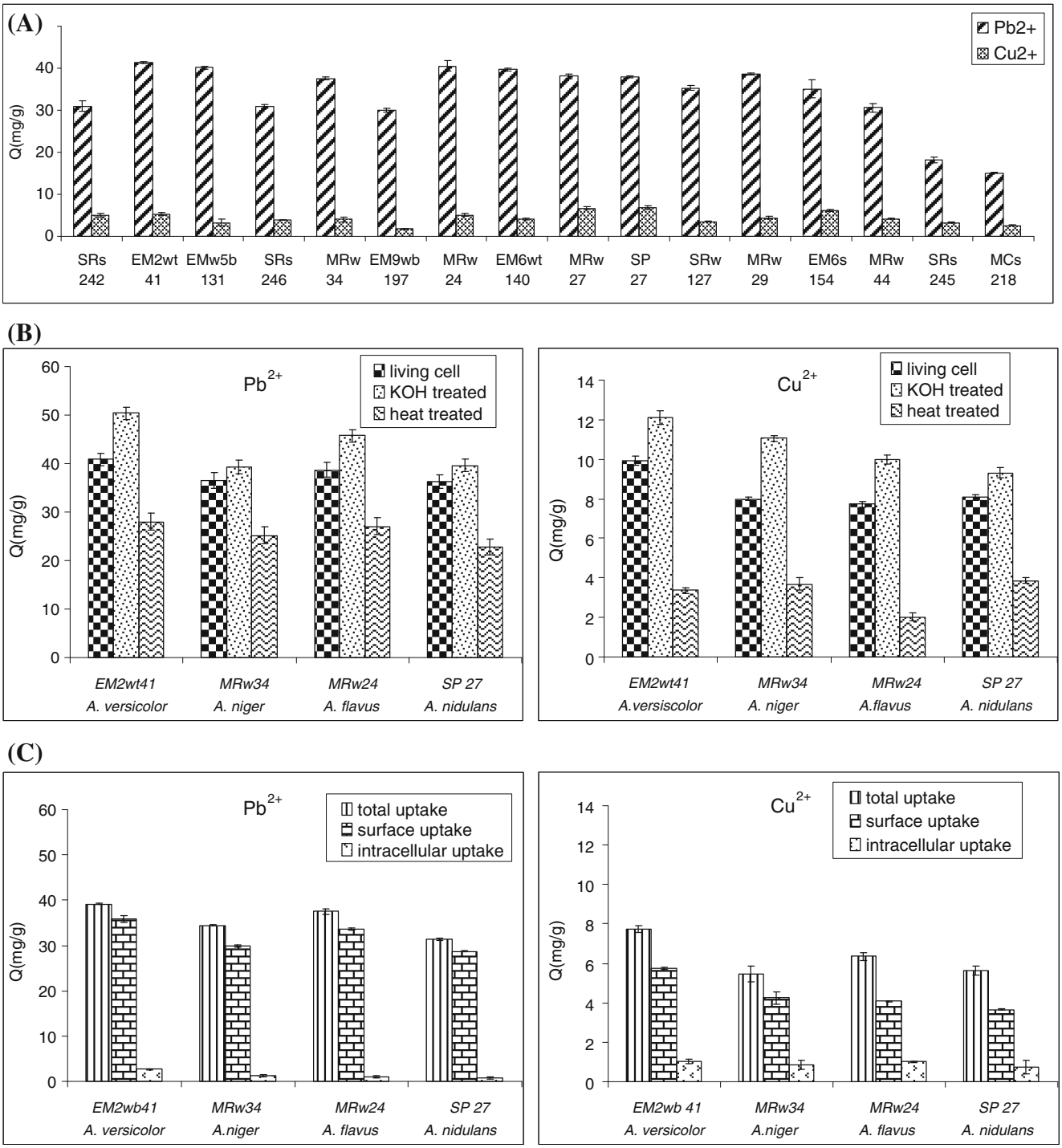

Fig. 2 Lead and Copper uptake (**a**) by living biomass of *Aspergillus* species; (**b**) by treated biomass; (**c**) Total, Cell-bound and Intracellular metal concentrations

Metal sorption by treated biomass

Four species that showed the highest metal sorption, namely, *Aspergillus versicolor* EM2wt41, *A. niger* MRw34, *A. flavus* MRw24 and *A. nidulans* SP27 were selected for study of effect of pretreatment of the biomass on metal sorption (Fig. 2B). It was observed that alkali pretreatment enhanced the sorption of metal while heat treatment decreased the sorption capacity, particularly of copper ions, in comparison to that by the native biomass. Amount of metal removal from solution was most by *A. versicolor*, followed by that by *A. flavus*, *A. nidulans* and *A. niger.*

The increased capacity in sorption of metals such as lead, copper, cadmium and chromium by alkali-treated mycelium of *Aspergillus fumigatus*, *A. niger*, *A. flavus* (Kapoor et al. 1999; Akar et al. 2007; Das et al. 2007; Al-Garni et al. 2009) as well as by other genera of *Cladosporium* sp., *Fusarium solani*, *Mucor rouxii*, *Rhizopus oryzae*, *Pleurotus florida* and *Saccharomyces uvarum* (Ashkenazy et al. 1997; Kowshik and Nazareth, 1999; Yan and Viraraghavan 2000; Baik et al. 2002; Xinjio 2006; Das et al. 2007). The enhanced metal-binding capacity by alkali pretreatment could be due to a deacetylation of chitin to chitosan, having an amine group involved in metal sorption (Akhtar et al. 1996; Guibal et al. 1995), a change also in the structure of dextran, or by dissociation of H^+ ions from the cell wall, resulting in the increase of negative functional groups (Xinjio 2006). It could be also due to binding of K^+ ions on the cell wall as analyzed by energy-dispersive X-ray analyzer, which then play a role in ion-exchange in metal binding involving a replacement of the K^+ ions adsorbed on the biosorbent, with the Pb^{2+} and Cu^{2+} ions (Gazem and Nazareth 2012).

The reduction in metal sorption by heat-treated mycelia corroborates the earlier report on *Aspergillus versicolor* (Cabuk et al. 2005). The dry heat treatment of the biomass could have adversely affected some of the biomolecules involved in metal biosorption, thus giving a lowered sorption capacity. It has been suggested that the heat treatment could cause a loss of amino-functional groups on the fungal surface through the non-enzymic browning reaction (Whistler and Daniel 1985). In contrast, Akar and Tunali (2006) reported that pretreatment of *A. flavus* biomass at 60 °C overnight increased the biosorption in comparison with native biomass.

These isolates were also found to tolerate salt to a level of 20-50‰ solar salt.

Cell-bound and intracellular metal uptake

The total amount of metal sorbed by the four selected cultures, the quantification of the metal bound to the cell surface and the intracellular accumulation are presented in Fig. 2C. Most of the metal removed from solution was sorbed on to the cell surface: 85.78 % - 94.77 % Pb^{2+} and 51.03 % - 77.51 % of Cu^{2+}, while only 2.35 % - 6.74 % lead and 11.17 % - 15.40 % Cu^{2+} was taken up intracellular. These results substantiate earlier work on *Corollospora lacera* (Taboski et al. 2005) wherein 93.6–95.6 % of the total Pb^{2+} was bound to the cell wall and only 1.7–5.5 % was found intracellularly, and with *Trichoderma viride* (Anand et al. 2006) in which 80–85 % was localized in the cell wall fraction and 9.0 % could be detected in the intracellular cytosol fraction; these values are higher than that of 47-64 % obtained for zinc removal by *Trichoderma atroviride* (Yazdani et al. 2010). The accumulation of lead granules on the outer layer of the cell wall of *Penicillium* species were also reported (Sun and Shao 2007).

FTIR

FTIR analysis (Fig. 3) of the biomass after metal sorption in comparison to that of the mycelial control before sorption, showed that there was change in the spectra with a broadening of bands and a shift or an increase in sharpness and intensity of peaks, that could be assigned to functional groups of biomolecules of the mycelium. The IR spectra

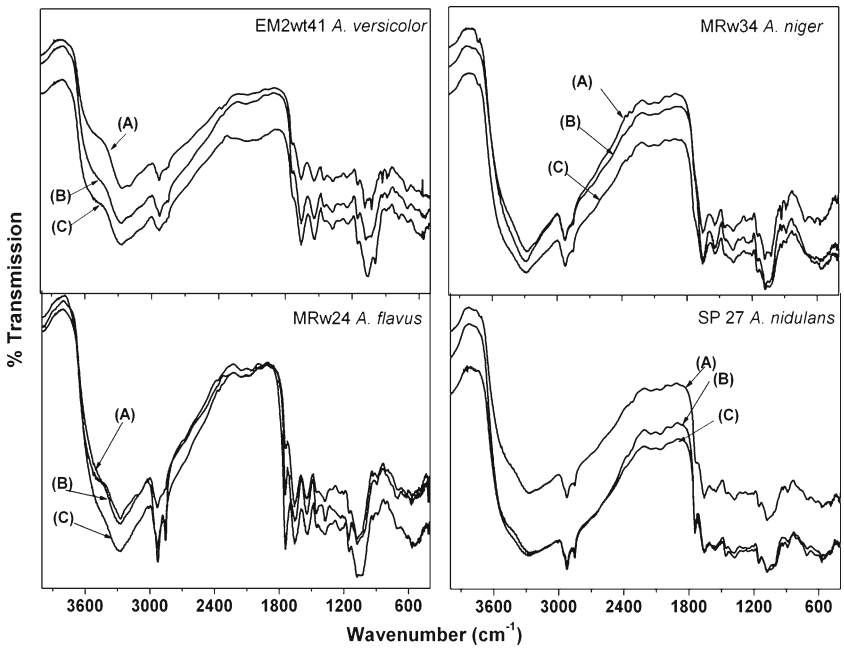

Fig. 3 IR spectra of *Aspergillus* species: mycelial biomass (**a**); mycelia after sorption with lead (**b**) and with copper (**c**)

of the biomass of *Aspergillus versicolor* EM2wt41 revealed a broadening of the -NH and -OH band at 3500-3200 cm^{-1}, of the –CH stretching vibrations of CH_2 and CH_3 groups at 3000-2800 cm^{-1}, a shift in peaks of the carbonyl –C = O of amide or carboxyl groups at 1670-1650 cm^{-1} and 1550-1540 cm^{-1}, the amide or sulfamide at 1381 cm^{-1}, the C-O or C-N stretching vibrations of proteins at 1153 cm^{-1} and the P-O-C linkage of the organo-phosphorous groups about 1026 cm^{-1}. It was observed that the changes were more pronounced when the biomass was challenged by Cu^{2+} than with Pb^{2+} ions, the peaks of carbonyl groups broadening and that of the phosphate group becoming sharper and more intense.

The IR spectra of the other three selected aspergilli biomass in comparison to that of *A. versicolor*, showed similar changes, with few variations. There was a greater broadening of the hydroxyl and amine band of *A. niger* MRw34 biomass challenged with metal, which was more pronounced in the case of *A. nidulans* SP27. Similarly, the –CH stretching vibrations at 2852 cm^{-1} were more prominent with the metal-loaded biomass of *A. flavus* MRw24 and *A. nidulans* SP27, and a shift in the peak of C = O stretching of esters at 1739.79 cm^{-1} in the mycelial control of *A. flavus* MRw24, to 1743.65 cm^{-1}, with greater sharpness and intensity, when challenged with Pb^{2+} and with Cu^{2+}, the latter being more prominent.

The results indicate the involvement of functional groups such as carboxylate, hydroxyl, sulfate, phosphate and amino groups present on the cell surface, in interaction with metals and subsequent removal of the ions from solution, that of hydroxyl and amine, together with the alkali and organo-phosphorous groups being more prominent. These observations support earlier results obtained on metal sorption by aspergilli: *Aspergillus niger*, *A. parasiticus*, *A. wentii* and *A. versicolor* (Akhtar et al. 1996; Akar et al. 2007; Acemioğlu et al. 2010; Bairagia et al. 2011). These biochemical groups would be mainly of the carbohydrate and protein molecules, and perhaps also the lipid moieties of the cell surface, corroborating earlier findings that the cell surface polysaccharide, proteins and chitins play a major role in metal sorption (Gadd 1993; Ashkenazy et al. 1997; Zafar et al. 2007), which would be responsible for the high percentage removal of metals from solution.

The FTIR analysis indicating the involvement of the functional groups of the cell surface in metal sorption substantiates the finding that most of the metal removed from solution was cell-bound. The slight variations between the IR spectra of the metal loaded biomass of the species, could be a reflection of the inter-species differences in the percentage of metal sorbed to the cell-surface.

This comparative study of different species establishes that metal sorption by aspergilli occurs mainly by sorption to the cell-wall, involving the functional groups of biomolecules of the cell surface. This mechanism is common to the different species of *Aspergillus;* however, variations were evidenced according to the fungal species, with *A. versicolor* giving the highest efficiency amongst the species tested.

These marine-derived fungal cultures show a good tolerance and a high sorptive capacity of heavy metals, making them potential biosorbents for removal of heavy metals from aqueous phase. They are also able to grow and withstand a salinity of 50‰; this characteristic gives an enhanced benefit for use of the isolates in bioremediation of metal pollution in saline systems.

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