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A comparative study of metal tolerance and sorption capacities of varied fungal genera from metal - polluted estuarine environments for potential in metal bioremediation

Akshaya Arjun Bicholkar and Sarita W. Nazareth*
Department of Microbiology, Goa University, Taleigao Plateau, Goa- 403206
Corresponding author Email: sarita@unigoa.ac.in
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ABSTRACT

Metal contaminated sites are known to support the development of metal resistant microorganisms, which can serve as biomonitors of a particular pollutant, and as agents for bioremediation. Fungal isolates were obtained from various metal-contaminated estuarine environments and assessed for their tolerance levels and sorption of the different heavy metals and transition metals. A comparative analysis of metal tolerance and sorption capability by fungi with respect to the various sites, the genera or species isolated, and their response to different metals showed that despite of high levels of a particular metal in a given econiche, a fungal organism may or may not have a high tolerance and/or sorption to the metal, and may therefore not necessarily be an indicator of metal pollution. Furthermore, fungal-metal interaction, while being more pronounced in the genera of *Penicillium* and *Aspergillus* also did not show uniformity within a given genus or species. Moreover, metal tolerance and its sorption by a given isolate had no definite correlation, as metal tolerance by a given species was not indicative of the sorptive capacity of the fungus, and could therefore be genus or species specific, as well as metal specific.

Keywords: Fungi, estuarine habitats, metal pollution, metal tolerance, metal sorption.

INTRODUCTION

Metals are normal constituents of the earth's crust. Anthropogenic activity with constantly increasing industrialization has resulted in their release into the surroundings. Metals are not degradable, and therefore accumulate, resulting in ever increasing levels of pollution in the environment. Aquatic water bodies are increasingly being polluted by the disposal of effluents. Metal pollution in the marine ecosystem has become a serious threat, as they accumulate throughout the food chain, leading to serious ecological and health problems (Laws, 1993; Rehman *et al.*, 2008; Banik *et al.*, 2013).

Bioremediation is an ecofriendly and cost-effective alternate technology that uses metabolic processes to degrade or transform contaminants, so that they remain no longer in harmful form. Fungi are preferred microorganisms for bioremediation processes because of their large biomass production and ease of biomass separation. They are a versatile group, able to adapt and grow under extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations, with the advantage of excellent metal-binding properties of their cell wall and capacity for efficient metal sequestration (Gadd, 1994; Volesky and Holan, 1995; Kapoor *et al.*, 1999; Baldrian and Gabriel, 2002; Singh, 2006; Wang and Chen, 2006; Yan and Viraraghavan, 2008; Gazem and Nazareth, 2013; Fomina and Gadd, 2014).

Organisms in metal contaminated sites, develop resistance to metals, and serve as agents for bioremediation (Gadd, 1994). In this context, a study was made on natural systems exposed to the metal contamination from iron ore mining industry. About two-thirds of the mining activities in Goa are located in the Mandovi basin, on the West Coast of the Indian peninsula. The Mandovi estuary connects with the Arabian Sea, and serves as a waterway for transport of the iron ore to the Mormugao harbor for the off-loading and export to eastern countries, particularly China, Japan, South Korea and Eastern European Countries. The estuary is also subjected to dumping of waste, including mine tailings (Alagarsamy, 2006; Mesquita and Kaisary, 2007; Ratnaprabha and Nayak, 2011).

In this work, it was sought to examine the capacity of fungi for metal tolerance and/or metal removal, whether is acquired in the presence of a pollutant metal in the environment, or is an inherent function of the biomass; whether it is enhanced in presence, or is independent of metal concentrations; and whether it is genus/species specific, or is definitive of a particular isolate.

MATERIALS AND METHODS

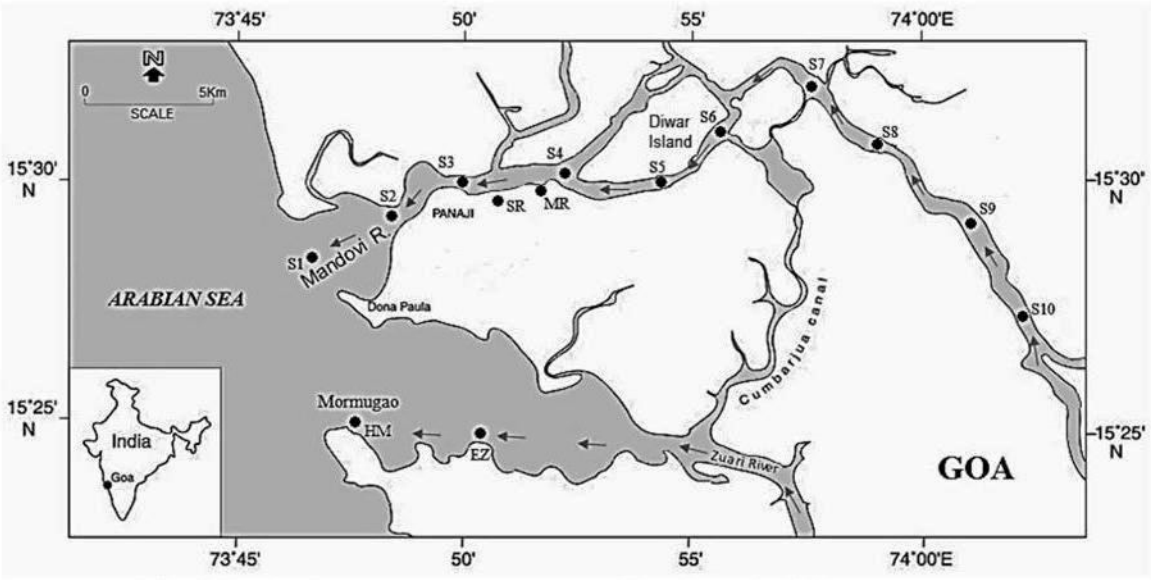
1) Sample sites and collection: Water and sediment samples were collected from five locations in Goa - India (**Fig. 1.**), during pre-monsoon season in the summer month of May.

Sampling was done from Mangroves at Ribander (MR) 15° 30'20.2"N, 73° 53.41"E. Water (w) (MRw) and sediment (s) samples (MRs) were collected aseptically from five sites, for microbial analysis, then pooled together. Similarly, brine water and sediment samples (SRw and SRs) were collected from solar Salterns at Ribander (SR), 15° 30'08.8"N, 73° 51.77"E, pooled together from five different saltpans at the time of salt harvesting.

Sampling was also carried out along the Mandovi estuary (EM), at ten stations: S1 to S10, beginning at the mouth and moving hinterland between 15° 28'34.1"N, 73° 46.65"E to 15° 27'59.8"N, 74° 02.05"E. Surface and bottom water samples, w_s and w_b, respectively, were collected with a Niskin water sampler and sediment using Grab sediment sampler; samples were labeled EM1w_s, EM1w_b and EM1s at station 1; and samples from stations 2-10 were labeled in similar manner.

Water and sediment samples were also obtained from the Zuar dock at Zuari Estuary (EZ) 15° 24'07.8"N, 73° 50.65"E, (EZw and EZs). Likewise, water samples were collected from the harbor at Mormugao (HMw) 15° 24'16.7"N, 73° 47.58"E, before and after the monsoons.

Water samples from the above mentioned sites were also collected in acid-washed polypropylene bottles for



MR : Ribander Mangrove
EM : Estuary, MandoviS1,S2..S10
S3 : Panjim ferry point (EM3)
S5 : Diwar ferry point (EM5)
SR : Ribander Saltern
EZ : Estuary, Zuar Dockyard
HM : Mormugao Harbor
:Barge movement

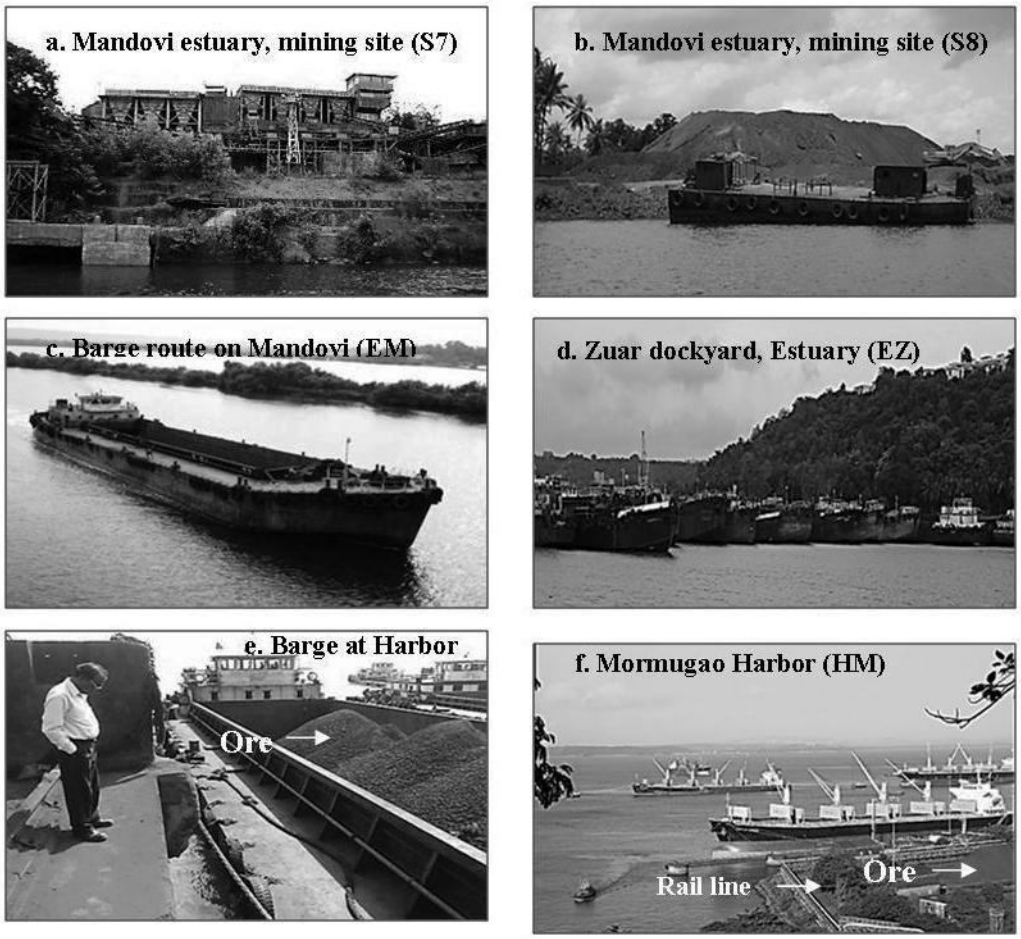


Fig.1. Sampling sites in Goa, India

analysis of metal content, and acidified with 5 ml 1% HNO_3 to eliminate reaction of any organic matter present. Samples were stored at 4 °C till they could be processed.

2) Assessment of levels of metals present in water samples: Metal concentrations in the water samples were measured by a modified solvent extraction method (Satyanarayanan, 2007). Water samples in duplicates, 100 ml each, along with a blank of deionized water, were acidified with 0.5 ml of 0.02 M HNO_3, and the pH adjusted to 4.1 for Fe and 6.4 for Pb, Cu, Cd, Mn using 3 M ammonia / 2 M acetic acid buffer. To this, 1 ml of 1% Ammonium pyrolidine dithiocarbamate (HIMEDIA®) and 5ml of Methyl-isobutyl ketone (Qualigens®)were added, which serves to extract a wide range of dithiocarbamate-metal chelates; the mixture was shaken vigorously for 5 min and then allowed to stand for 20-30 min. To the upper organic phase, 20 ml of 4N HNO_3 was added and shaken for acid back-extraction. The lower aqueous phase was transferred to polypropylene tubes for further analysis by atomic absorption spectrophotometry (AAS), (Shimadzu AA-6300).

3) Isolation and identification of fungi: Water and sediment suspensions in 2% saline were spread plated on Czapek-Dox Agar (HIMEDIA®) + 2% NaCl termed as S-CzA, incubated at 30 °C for 2-4 days. The isolates were picked based on the dissimilarity in the colony characteristics, purified and maintained on S-CzA at 4 °C. The isolates were identified to the genus level on the basis of colony and microscopic characteristics with reference to standard fungal identification keys (Raper and Fennell, 1965; Ellis, 1971; Domsch *et al.*, 1980).

4) Metal tolerance: The isolates were screened for metal tolerance by spot inoculation of spore suspensions on S-CzA plates, each containing a single metal ion: 10 mM Pb^{2+} as $Pb(NO_3)_2$, 5 mM Cu^{2+} as $CuSO_4$, 2 mM Cd^{2+} as $Cd(NO_3)_2$ or as $CdSO_4$, 5 mM Fe^{3+} as $FeCl_3$, 10 mM Fe^{2+} as $FeSO_4$ and 50 mM Mn^{2+} as $MnSO_4$, and incubated at 30 °C for 4 days.

Tolerance index (TI) was determined as the ratio of the growth obtained on medium containing metal to that on medium without metal. The maximum tolerance concentration (MTC) was determined by spot-inoculating, in triplicate, on S-CzA containing metal in the range of 0-25 mM Pb^{2+} or Cu^{2+}; 0-6 mM Cd^{2+}as nitrate and as sulphate; 0-10 mM Fe^{3+}; 0-25 mM Fe^{2+}; 0-250 mM Mn^{2+}. The plates were incubated at 30 °C and growth was recorded in terms of colony diameter after 4 days, and examined visually up to 7 days for changes in growth pattern, sporulation and pigment production, compared against the control of isolates grown in the absence of the metal.

5) Metal Biosorption: Isolates were selected based on the criterion of belonging to different genera, isolated from different econiches and possessing a high tolerance to the metal(s). Spore suspension containing 10^6 spores, was inoculated in 100 ml of Czapek-Dox Broth + 2% NaCl (S-CzB) and incubated for 3 days at 30 °C, 150 rpm. The biomass was harvested by filtering through double layered muslin cloth and washed with 100 ml saline, then washed twice with deionized water. Aliquots of the biomass were taken to obtain the wet weight and then dried to constant weight at 60 °C to obtain the dry weight. Mycelial biomass, 1 g wet weight, approx. 0.1 g dry weight, was homogenized with a mortar and pestle, suspended in 20 ml of 1 mM of individual metal solution with pH adjusted to 6.0. Control flasks of only metal solution were maintained. The flasks were incubated at 150 rpm for 1 h at 30 °C and the biomass then filtered off through Whatman no. 1 filter paper.The filtrates, 10 ml, were digested with 50% concentrated HNO_3, 0.5 ml, for 10 min and then made up to original volume with deionized water. The metal concentration was analyzed by AAS. All the experiments were carried out in duplicates.

The amount of metal sorbed by the fungal biomass was calculated using the following equation: Q (mg l^{-1}) = V (C_i - C_f) /S, where Q = mg of metal ion sorbed g^{-1} dry weight biomass; C_i = initial metal ion concentration (mg l^{-1}); C_f = final metal ion concentration (mg l^{-1}); S = biosorbent (g), V = volume (l) of the reaction mixture (Volesky and Holan, 1995).

6) Statistical analysis of metal tolerance and sorbing capacity: The metal tolerance and sorption levels of the selected isolates were statistically analyzed for similarity or difference in sorption capacity between the genera and/or species. Statistical analysis was done using the online software Web Agri Stat Package-2.0 (WASP) designed by Indian Council of Agricultural Research, Goa, India.

RESULTS

Levels of metals present in water samples: The concentrations of heavy metals: lead, copper and cadmium as well as transition metals: iron and manganese in the water at the various sites of sampling are shown in **Fig. 2.** The levels of metals in the Mandovi estuary was examined at two Stations, Station 3, the Diwar ferry point (EM3) and Station 5, the Panjim ferry point (EM5). It was observed that iron concentrations at EM5, was only 0.16 ppm, while it was very high 5.4 ppm at EM3. In contrast, manganese was fairly high at 1.82-1.86 ppm in all the samples tested. At mangroves, lead and copper concentrations were not significantly high, but cadmium was high at 0.17 ppm. Saltern brine sample registered high concentration of 0.97 ppm cadmium, 9.91 ppm iron and 8.26 ppm manganese, but lead and copper being at lower concentrations. Zuar dock and Mormugao harbor showed extremely high levels of 4.34 ppm and 1.22 ppm, respectively, the highest lead contamination amongst the sites examined; cadmium levels were also high at these sites, at 0.26 ppm and 0.05 ppm, respectively.

Fungal isolates: Isolates obtained belonged predominantly to *Penicillium* and *Aspergillus*; the other genera obtained included *Fusarium* and the dematiaceous fungi, *Alternaria* and *Cladosporium*. The isolates, selected for study on the basis of their metal tolerance and representative species from different locations (**Table 1**), were tentatively identified as *Penicillium*

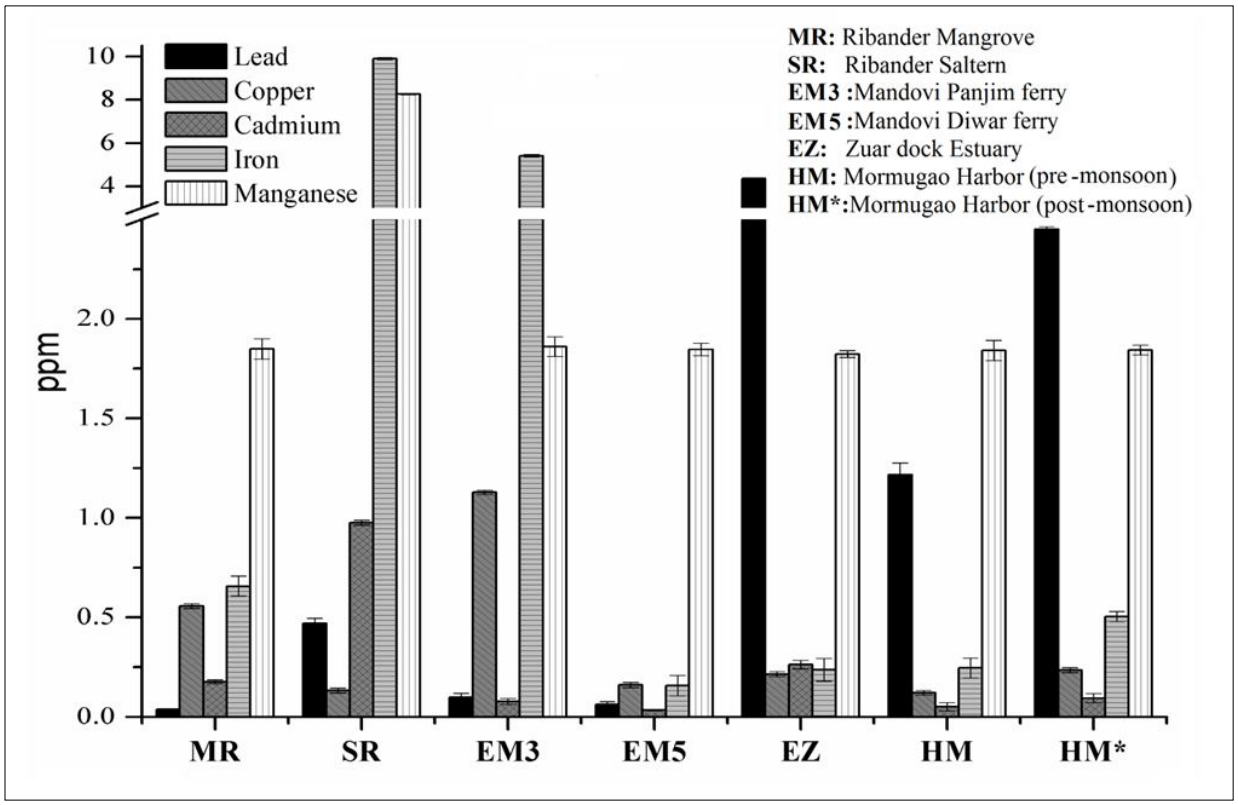


Fig.2. Levels of metals in water samples

brevicompactum, *P. citrinum*, *P. corylophilum*, *P. glabrum*; *Aspergillus flavus*, *A. flavus* var. *flavus*, *A. flavus* var *columnaris*, *A. niger*, *A. oryzae*, *A. terreus*, *A. versicolor*, *A. fumigatus*, *A. awamori*; *Fusarium chlamydosporum*, *F. oxysporum*, *F. solani*, *F. sporotrichioides*; *Alternaria alternata*, *A. citri*, *A. sonchi*; *Cladosporium cladosporioides*, *C. chlorocephalum*, *C. oxysporum*, *C. cucumerinum* and *C. spongiosum.*

Metal Tolerance

i) Screening for metal tolerance: A total of 147 isolates were screened for tolerance to selected metals: fifteen isolates from Ribander mangroves (MR) and fifteen from salterns (SR), fifty from Mandovi Estuary (EM), thirty-seven from Zuar-dock (EZ) and thirty from Mormugao harbor (HM). Tolerance index (TI) obtained (**Fig. 3.A, 3.B**) indicated that most of the isolates from MR and SR showed significant tolerance towards 10 mM lead, with most isolates of *Penicillium* having an index ≥ unity and *Aspergillus* index close to 1, whereas isolates from EM, EZ and HM had TI ≤ 1. Most isolates from all sites showed a moderate tolerance, with TI ≤ 1 for 5 mM copper and iron, and for 50 mM manganese. Many isolates showed TI < 0.5 for 2 mM cadmium with a few penicilli having TI ≤ 1. *Penicillium* species had a lower tolerance to cadmium nitrate as compared to cadmium sulphate. It was noted that all aspergilli from salterns that were examined, could not grow in presence of even 0.5 mM cadmium salts.

ii) Maximum metal tolerance concentrations: Seventy two isolates were selected based on their ability to grow in presence of high concentrations of heavy metals and transition metals, ten each from MR and SR, a total of thirty two from all the ten stations of EM, and ten each from EZ and HM, for determination of MTC of metals. The highest MTC of each metal by each genera (**Table 2.A, 2.B**) was shown by *Penicillium* and *Aspergillus* at 15 mM Pb^{2+}, 10 mM Cu^{2+}, 5 mM Cd^{2+} as nitrate, 7.5 mM Cd^{2+} as sulphate, 7.5 mM Fe^{3+}, 25 mM Fe^{2+} and 250 mM Mn^{2+}, while *Fusarium* and the dematiaceous fungi *Alternaria* and *Cladosporium* showed MTC values ranging from 7.5-10 mM Pb^{2+}, 7.5 mM Cu^{2+}, 2-4 mM Cd^{2+} as nitrate, 4-5 mM Cd^{2+} as sulphate, 5 mM Fe^{3+}, 7.5-20 mM Fe^{2+} and 100-200 mM Mn^{2+}. The statistical analysis indicated that the genera of *Penicillium* and *Aspergillus* have the maximum tolerance capacity as compared to the other genera.

There was dissimilarity in metal tolerance between the different species of *Penicillium*: *P. corylophilum, P. brevicompactum* and *P. glabrum*. *P. corylophilum* EM2w_b256 and *P. glabrum* EM1s239 possessed the maximum tolerance to lead, copper and ferrous ions, while *P. citrinum* SRw119 showed the highest tolerance to cadmium sulphate and both ferric and ferrous salts. In addition, while *P. corylophilum* showed the highest MTC of lead, *P. brevicompactum* showed the lowest MTC. This was also seen amongst different species of aspergilli, with *A. Oryzae* EM3s281 exhibiting the highest tolerance to copper, cadmium nitrate and manganese; however, similar metal tolerance levels by the isolated strains of *A. niger* were observed.

Table 1. Fungal isolates obtained from the various sampling sites

Site	Genus	Species	Isolate number
Ribander Mangrove	*Aspergillus*	*A. flavus* var. *flavus*	MRw4
	Penicillium	*P. brevicompactum*	MRw17, MRw19
		P. corylophilum	MRw1,MRw2,MRw3,MRw5, MRw6, MRw7, MRw9
Ribander Saltern	*Aspergillus*	*A. niger*	SRw108, SRw109, SRw110, SRw111, SRw112, SRw113, SRw114
		A. terreus	SRw115
	Penicillium	*P. citrinum*	SRw119
	Cladosporium	*C. chlorocephalum*	SRw120
Mandovi Estuary	*Aspergillus*	*A. flavus* var. *flavus*	EM5w$_s$317
		A. flavus var. *columnaris*	EM7w$_b$363
		A. niger	EM3s285
		A. oryzae	EM3s281, EM9w$_s$390
		A. terreus	EM1w$_b$234, EM9w$_b$398
		A. versicolor	EM4w$_s$294
		A. fumigatus	EM2w$_b$253
		A. awamori	EM10w$_s$408
	Penicillium	*P. corylophilum*	EM2w$_b$256, EM2s260, EM5w$_s$315, EM7s373, EM8w$_s$377, EM9s404
		P. glabrum	EM1s239, EM3w$_s$264, EM6w$_b$346
	Fusarium	*F. chlamydosporum*	EM9w$_s$392
		F. solani	EM1w$_s$210
		F. sporotrichioides	EM3w$_s$278, EM4w$_s$295
	Cladosporium	*C. cladosporioides*	EM1w$_b$232, EM8s385
		C. herbarum	EM4w$_s$288
		C. oxysporum	EM4w$_s$300, EM6w$_s$338
		C. cucumerinum	EM6w$_s$332
		C. spongiosum	EM10s423
	Alternaria	*A. alternata*	EM7s367
		A. sonchi	EM8s388
Zuar dock Zuari Estuary	*Aspergillus*	*A. flavus*	EZs526
		A. terreus	EZw501
	Penicillium	*P. brevicompactum*	EZs530
		P. glabrum	EZw514
	Fusarium	*F. oxysporum*	EZw520, EZs534
	Cladosporium	*C. chlorocephalum*	EZs531
		C. oxysporum	EZw507
	Alternaria	*A. alternata*	EZw524
		A. citri	EZw523
Mormugao Harbor	*Aspergillus*	*A. niger*	HMw605
		A. versicolor	HMw602
	Penicillium	*P. brevicompactum*	HMw617
		P. corylophilum	HMw626
	Fusarium	*F. oxysporum*	HMw603, HMw611
	Cladosporium	*C. cladosporioides*	HMw610, HMw621
	Alternaria	*A. alternata*	HMw601
		A. citri	HMw615

Table 2.A. Statistical analysis of tolerance and sorption of heavy metals by the isolates

Isolate No.	Species	MTC Pb^{2+} mM	Q (mg g^{-1}) Pb^{2+}	MTC Cu^{2+} mM	Q (mg g^{-1}) Cu^{2+}	MTC Cd^{2+} $(NO_3)_2$ mM	Q (mg g^{-1}) Cd^{2+}	MTC Cd^{2+} SO_4 mM	Q (mg g^{-1}) Cd^{2+}
MRw1	*Penicillium corylophilum*	^{c} 10.0	^{k} 32.29±0.20	^{f} 2.5	^{k} 2.24±0.07	^{c} 2.0	^{nopq} 0.38±0.10	^{f} 2.0	^{s} 4.15±0.02
MRw2	*P. corylophilum*	^{a} 15.0	^{h} 36.28±0.01	^{c} 5.0	^{k} 2.09±0.18	^{c} 2.0	^{mnop}0.50±0.06	^{c} 5.0	^{v} 1.02±0.05
MRw3	*P. corylophilum*	^{b} 12.5	^{z} 12.17±0.20	^{c} 5.0	^{mn}1.50±0.08	^{c} 2.0	^{k} 1.57±0.03	^{c} 5.0	^{u} 1.66±0.03
MRw4	*Aspergillus versicolor*	^{a} 15.0	^{k} 32.28±0.07	^{f} 2.5	^{h} 3.74±0.08	^{e} 0.5	^{l} 0.91±0.24	^{e} 3.0	^{u} 1.33±0.04
MRw5	*Penicillium corylophilum*	^{c} 10.0	^{a} 45.03±0.04	^{c} 5.0	^{k} 2.30±0.24	^{d} 1.0	^{h} 3.54±0.01	^{c} 5.0	^{x} 0.15±0.05
MRw6	*P. corylophilum*	^{b} 12.5	^{z} 16.17±0.10	^{c} 5.0	^{j} 2.86±0.23	^{c} 2.0	^{t} 0.02±0.24	^{f} 2.0	^{op} 5.99±0.01
MRw7	*P. corylophilum*	^{b} 12.5	^{q} 27.24±0.14	^{c} 5.0	^{j} 2.97±0.02	^{e} 0.5	^{r} 0.18±0.02	^{e} 3.0	^{x} 0.08±0.01
MRw9	*P. corylophilum*	^{b} 12.5	^{c} 40.05±0.05	^{c} 5.0	^{i} 3.00±0.03	^{c} 2.0	^{r} 0.19±0.04	^{a} 7.5	^{x} 0.08±0.04
MRw17	*P. brevicompactum*	^{d} 7.5	^{r} 26.81±0.81	^{f} 2.5	^{h} 3.83±0.04	^{e} 0.5	^{s} 0.15±0.03	^{e} 3.0	^{x} 0.23±0.03
MRw19	*P. brevicompactum*	^{e} 5.0	^{y} 18.32±0.60	^{f} 2.5	^{h} 3.80±0.04	^{h} 0.0	^{lm}0.80±0.08	^{a} 7.5	^{x} 0.14±0.02
SRw108	*Aspergillus niger*	^{e} 5.0	^{v} 22.86±0.61	^{c} 5.0	^{g} 4.02±0.12	^{h} 0.0	^{k} 1.50 ±0.04	^{h} 0.0	^{x} 0.23±0.01
SRw109	*A. niger*	^{e} 5.0	^{x} 20.92±0.14	^{c} 5.0	^{lm}1.76±0.09	^{h} 0.0	^{lmn}0.73±0.01	^{h} 0.0	^{x} 0.19±0.04
SRw110	*A. niger*	^{e} 5.0	^{i} 34.64±0.01	^{c} 5.0	^{lm}1.67±0.06	^{h} 0.0	^{l} 0.89±0.01	^{h} 0.0	^{u} 1.43±0.02
SRw111	*A. niger*	^{e} 5.0	^{s} 24.95±0.11	^{c} 5.0	^{l} 1.83±0.07	^{h} 0.0	^{l} 1.06±0.07	^{h} 0.0	^{w} 0.69±0.04
SRw112	*A. niger*	^{e} 5.0	^{u} 30.06±0.30	^{c} 5.0	^{j} 2.87±0.01	^{h} 0.0	^{opq}0.34±0.08	^{h} 0.0	^{x} 0.11±0.07
SRw113	*A. niger*	^{e} 5.0	^{z} 13.60±0.17	^{c} 5.0	^{n} 1.39±0.01	^{h} 0.0	^{l} 1.00±0.03	^{h} 0.0	^{x} 0.11±0.06
SRw114	*A. niger*	^{c} 10.0	^{b} 40.66±0.12	^{c} 5.0	^{mn}1.78±0.19	^{h} 0.0	^{l} 1.04±0.08	^{h} 0.0	^{u} 1.56±0.09
SRw115	*A. terreus*	^{c} 10.0	^{e} 38.02±0.06	^{f} 2.5	^{j} 2.81±0.14	^{h} 0.0	^{g} 4.02±0.06	^{h} 0.0	^{r} 4.52±0.07
SRw119	*Penicillium citrinum*	^{b} 12.5	^{a} 45.10±0.01	^{c} 5.0	^{b} 6.24±0.01	^{a} 4.0	^{f} 5.18±0.01	^{a} 7.5	^{q} 5.02±0.01
SRw120	*Cladosporium chloro-cephalum*	^{e} 5.0	^{j} 33.81±0.09	^{f} 2.5	^{d} 4.91±0.15	^{b} 3.0	^{j} 2.50±0.06	^{c} 5.0	^{t} 3.04±0.07
EM2w_b253	*Aspergillus fumigatus*	^{b} 12.5	^{op} 28.69±0.2	^{a} 10.0	^{op} 0.95±0.03	^{b} 3.0	^{e} 7.71±0.2	^{e} 3.0	^{e} 39.3±0.1
EM2w_b256	*Penicillium corylophilum*	^{a} 15.0	^{k} 32.69±0.01	^{a} 10.0	^{ef}4.24±0.06	^{a} 4.0	^{l} 2.72±0.08	^{b} 6.0	^{l} 31.1±0.07
EM4w_s288	*Cladosporium herbarum*	^{c} 10.0	^{m} 30.58±0.04	^{d} 4.0	^{ef}4.49±0.06	^{b} 3.0	^{k} 1.82± 0.04	^{a} 7.5	^{h} 36.26±0.04
EM6w_s332	*C. cucumerinum*	^{c} 10.0	^{z} 8.58±0.19	^{b} 7.5	^{s} 0.49±0.05	^{b} 3.0	^{k} 1.82±0.07	^{c} 5.0	^{h} 36.26±0.35
EM8s388	*Alternaria sonchi*	^{e} 5.0	^{b} 40.69±0.07	^{c} 5.0	^{c} 5.57±0.04	^{c} 2.0	^{ij}2.92±0.13	^{f} 2.0	^{j} 33.44±0.22
EM9w_s392	*Fusarium chlamy-dosporum*	^{e} 5.0	^{f} 37.44±0.04	^{c} 5.0	^{s} 0.40±0.01	^{e} 0.5	^{b}15.39±0.01	^{g} 1.0	^{a} 46.76±0.06
EZw514	*Penicillium glabrum*	^{b} 12.5	^{z} 12.58±0.09	^{c} 5.0	^{e} 4.40±0.03	^{c} 2.0	^{k} 1.74±0.41	^{c} 5.0	^{k} 32.36±0.68
EZw520	*Fusarium oxysporum*	^{e} 5.0	^{t} 23.65±0.14	^{e} 3.0	^{r} 0.65±0.03	^{d} 1.0	^{b} 15.8±0.38	^{e} 3.0	^{c} 42.24±0.27
EZw524	*Alternaria alternata*	^{d} 7.5	^{gh}36.46±0.04	^{e} 3.0	^{e} 4.53±0.04	^{d} 1.0	^{f} 5.29±0.57	^{e} 3.0	^{g}37.28±0.27
EZs526	*Aspergillus flavus*	^{c} 10.0	^{u} 23.22±0.81	^{d} 4.0	^{s} 0.44±0.03	^{d} 1.0	^{k} 1.91±0.37	^{d} 4.0	^{k} 32.68±0.1
EZs531	*Cladosporium chloro-cephalum*	^{e} 5.0	^{z} 14.73±0.61	^{e} 3.0	^{t} 0.31±0.03	^{d} 1.0	^{d} 11.46±0.43	^{d} 4.0	^{d} 39.58±0.84
HMw601	*Alternaria alternata*	^{c} 10.0	^{d} 39.27±0.61	^{c} 5.0	^{a} 7.73±0.06	^{c} 2.0	^{f} 5.27±0.29	^{c} 5.0	^{i} 35.91±0.19
HMw602	*Aspergillus versicolor*	^{d} 7.5	^{z} 17.33±0.14	^{c} 5.0	^{t} 0.34±0.03	^{d} 1.0	^{j} 2.3±0.48	^{e} 3.0	^{m} 26.22±1.14
HMw610	*Cladosporium cladosporioides*	^{e} 5.0	^{l} 31.04±0.01	^{e} 3.0	^{s} 0.47±0.03	^{d} 1.0	^{f} 5.63±0.12	^{e} 3.0	^{f} 38.12±0.06
HMw611	*Fusarium oxysporum*	^{d} 7.5	^{w} 21.37±0.11	^{c} 5.0	^{q} 0.51±0.03	^{c} 2.0	^{a} 16.78±0.07	^{e} 3.0	^{b} 42.76±0.61
HMw615	*Alternaria citri*	^{d} 7.5	^{g} 36.61±0.06	^{c} 5.0	^{e} 4.55±0.03	^{c} 2.0	^{f} 5.26±0.08	^{c} 5.0	^{g}37.25± 0.05
HMw626	*Penicillium corylophilum*	^{c} 10.0	^{r} 26.47±0.29	^{c} 5.0	^{fg}4.14±0.07	^{c} 2.0	^{h} 3.16±0.01	^{c} 5.0	^{n} 25.76±0.65

$^{a-z}$: denotes statistical 'demarcation of ANOVA values'; ± : Standard Error

There was a dissimilarity in metal tolerance seen also amongst species of each of the other genera, *Fusarium, Alternaria* and *Cladosporium*:*Fusarium oxysporum* HMw611 showed the highest tolerance to 3 metals, namely, lead, copper and ferrous ions; *Alternaria alternata* HMw601 had the greatest tolerance to lead, copper and cadmium salts, and *Alternaria citri* HMw615 to copper, cadmium salts and ferrous ions; *Cladosporium herbarum* EM4w_s288 was most tolerant of lead, copper, cadmium sulphate and ferrous sulphate. However, a similarity in metal tolerance was also observed between two different species of *Alternaria,* namely *A. alternata* HMw601 and *A. citri* HMw615, obtained from the same site at harbor.

Table 2.B. Statistical analysis of tolerance and sorptionof transitional metals by the isolates

Isolate No.	Species	MTC Fe^{3+}	Q (mg g^{-1}) Fe^{3+}	MTC Fe^{2+}	Q (mg g^{-1}) Fe^{2+}	MTC Mn^{2+}	Q (mg g^{-1}) Mn^{2+}
MRw1	*Penicillium corylophilum*	^{b}5.0	^{hi}10.47±0.02	^{f}5.0	^{e}16.54±0.18	^{e}75.0	^{i}32.15±0.01
MRw2	*P. corylophilum*	^{a}7.5	^{ef}10.76±0.04	^{f}5.0	^{a}20.12±0.07	^{e}75.0	^{f}35.56±0.05
MRw3	*P. corylophilum*	^{b}5.0	^{hi}10.44±0.06	^{b}20.0	^{a}20.11±0.06	^{d}100.0	^{g}34.18±0.08
MRw4	*Aspergillus versicolor*	^{b}5.0	^{fg}10.65±0.01	^{b}20.0	^{a}20.08±0.02	^{e}75.0	^{i}32.23±0.08
MRw5	*Penicillium corylophilum*	^{b}5.0	^{hi}10.46±0.01	^{a}25.0	^{b}19.99±0.02	^{f}50.0	^{a}43.70±0.05
MRw6	*P. corylophilum*	^{b}5.0	^{d}10.93±0.08	^{d}10.0	^{a}20.58±0.02	^{f}50.0	^{b}42.35±0.03
MRw7	*P. corylophilum*	^{a}7.5	^{hi}10.41±0.01	^{d}10.0	^{b}19.94±0.03	^{e}75.0	^{f}35.73±0.06
MRw9	*P. corylophilum*	^{a}7.5	^{l}10.09±0.04	^{d}10.0	^{a}20.28±0.01	^{e}75.0	^{c}41.36±0.02
MRw17	*P. brevicompactum*	^{a}7.5	^{gh}10.50±0.02	^{c}15.0	^{a}20.38±0.02	^{d}100.0	^{h}33.87±0.04
MRw19	*P. brevicompactum*	^{a}7.5	^{jk}10.29±0.05	^{c}15.0	^{a}20.53±0.04	^{d}100.0	^{f}35.91±0.03
SRw108	*Aspergillus niger*	^{a}7.5	^{ef}10.73±0.01	^{c}15.0	^{a}20.69±0.02	^{e}75.0	^{e}36.47±0.02
SRw109	*A. niger*	^{a}7.5	^{kl}10.14±0.09	^{b}20.0	^{b}19.65±0.01	^{d}100.0	^{d}37.21±0.01
SRw110	*A. niger*	^{a}7.5	^{gh}10.56±0.04	^{c}15.0	^{a}20.31±0.07	^{e}75.0	^{h}33.30±0.12
SRw111	*A. niger*	^{b}5.0	^{s}6.62±0.06	^{f}5.0	^{a}20.65±0.02	^{f}50.0	^{a}43.70±0.02
SRw112	*A. niger*	^{b}5.0	^{c}11.26±0.02	^{d}10.0	^{c}18.84±0.02	^{f}50.0	^{g}34.84±0.08
SRw113	*A. niger*	^{a}7.5	^{de}10.84±0.02	^{d}10.0	^{a}20.57±0.08	^{d}100.0	^{h}33.68±0.06
SRw114	*A. niger*	^{a}7.5	^{c}11.14±0.03	^{b}20.0	^{a}20.52±0.02	^{d}100.0	^{a}43.00±0.01
SRw115	*A. terreus*	^{b}5.0	^{m}9.82±0.03	^{c}15.0	^{e}16.66±0.37	^{f}50.0	^{e}36.31±0.70
SRw119	*Penicillium citrinum*	^{a}7.5	^{a}12.56±0.15	^{a}25.0	^{d}17.77±0.38	^{d}100.0	^{a}43.93±0.02
SRw120	*Cladosporium chlorocephalum*	^{f}2.0	^{c}11.19±0.01	^{f}5.0	^{e}16.84±1.02	^{g}25.0	^{b}42.82±0.01
EM2w$_b$253	*Aspergillus fumigatus*	^{b}5.0	^{ij}10.3±0.01	^{b}20.0	^{m}6.57±0.07	^{d}100.0	^{m}9.82±0.02
EM2w$_b$256	*Penicillium corylophilum*	^{c}4.0	^{b}11.48±0.01	^{a}25.0	^{l}7.77±0.03	^{a}200.0	^{q}8.84±0.03
EM4w$_s$288	*Cladosporium herbarum*	^{c}4.0	^{nop}9.19 ± 0.04	^{e}7.5	^{h}10.98±0.04	^{e}75.0	^{mn}9.74± 0.04
EM6w$_s$332	*C. cucumerinum*	^{d}3.0	^{nop}9.19±0.07	^{f}5.0	^{gh}10.98±0.01	^{f}50.0	^{mn}9.74±0.04
EM8s388	*Alternaria sonchi*	^{f}2.0	^{c}11.48±0.01	^{g}2.5	^{m}6.07±0.03	^{e}75.0	^{m}9.69±0.06
EM9w$_s$392	*Fusarium chlamydosporum*	^{g}0.5	^{r}6.97±0.07	^{g}2.5	^{kl}7.61±0.01	^{g}25.0	^{jkl}10.74±0.02
EZw514	*Penicillium glabrum*	^{a}7.5	^{b}11.42±0.03	^{a}25.0	^{l}7.26±0.06	^{b}150.0	^{q}8.96±0.06
EZw520	*Fusarium oxysporum*	^{e}2.5	^{r}7.02±0.003	^{g}2.5	^{op}2.78±0.03	^{f}50.0	^{kl}10.58±0.02
EZw524	*Alternaria alternata*	^{b}5.0	^{c}11.48±0.01	^{g}2.5	^{I}9.40±0.01	^{c}125.0	^{l}10.66±0.01
EZs526	*Aspergillus flavus*	^{a}7.5	^{n}9.24±0.01	^{c}15.0	^{j}8.40±0.03	^{c}125.0	^{opq}8.86±0.01
EZs531	*Cladosporium chlorocephalum*	^{b}5.0	^{q}8.13±0.01	^{d}10.0	^{I}9.44±0.01	^{g}25.0	^{l}10.67±0.02
HMw601	*Alternaria alternata*	^{b}5.0	^{b}11.48±0.03	^{g}2.5	^{f}11.14±0.01	^{e}75.0	^{j}11.14±0.02
HMw602	*Aspergillus versicolor*	^{a}7.5	^{r}7.03±0.03	^{b}20.0	^{n}5.42±0.07	^{d}100.0	^{nop}9.25±0.03
HMw610	*Cladosporium cladosporioides*	^{e}2.5	^{t}5.90±0.01	^{f}5.0	^{jk}8.16±0.03	^{f}50.0	^{kl}10.77±0.07
HMw611	*Fusarium oxysporum*	^{e}2.5	^{op}9.1±0.01	^{c}15.0	^{l}7.73±0.04	^{g}25.0	^{jk}10.92±0.04
HMw615	*Alternaria citri*	^{b}5.0	^{b}11.46±0.04	^{d}10.0	^{i}9.20±0.07	^{e}75.0	^{kl}10.36± 0.05
HMw626	*Penicillium corylophilum*	^{a}7.5	^{b}11.44±0.01	^{b}20.0	^{fg}11.10±0.01	^{c}125.0	^{q}8.86±0.02

$^{a-z}$: denotes statistical 'demarcation of ANOVA values'; ± : Standard Error

iii) Morphological changes in growth in response to metals: Growth of the fungal isolates was inhibited to a very slight extent in presence of lower concentrations of metals, other than the cadmium salts examined, cadmium nitrate being most inhibitory. However, when grown in presence of higher metal concentrations, the isolates grew slower, and showed striking variations in colony appearance as compared to the controls, becoming increasingly compact in presence of copper and cadmium, or with mycelia thinning out at the growing edge in presence of lead, iron and manganese, and a delayed sporulation. The colony of *Penicillium*, *Alternaria* and *Cladosporium* isolates grown in presence of copper and manganese, showed an undulate surface, while all isolates grown in presence of cadmium nitrate had an irregular margin and a crenate surface.

Aspergillus and *Fusarium* species yielded a zone of clearance around the colony, in response to lead, copper, cadmium and manganese but not to iron salts.

Pigment production in *Penicillium*, *Alternaria* and *Cladosporium* was enhanced in response to metals other

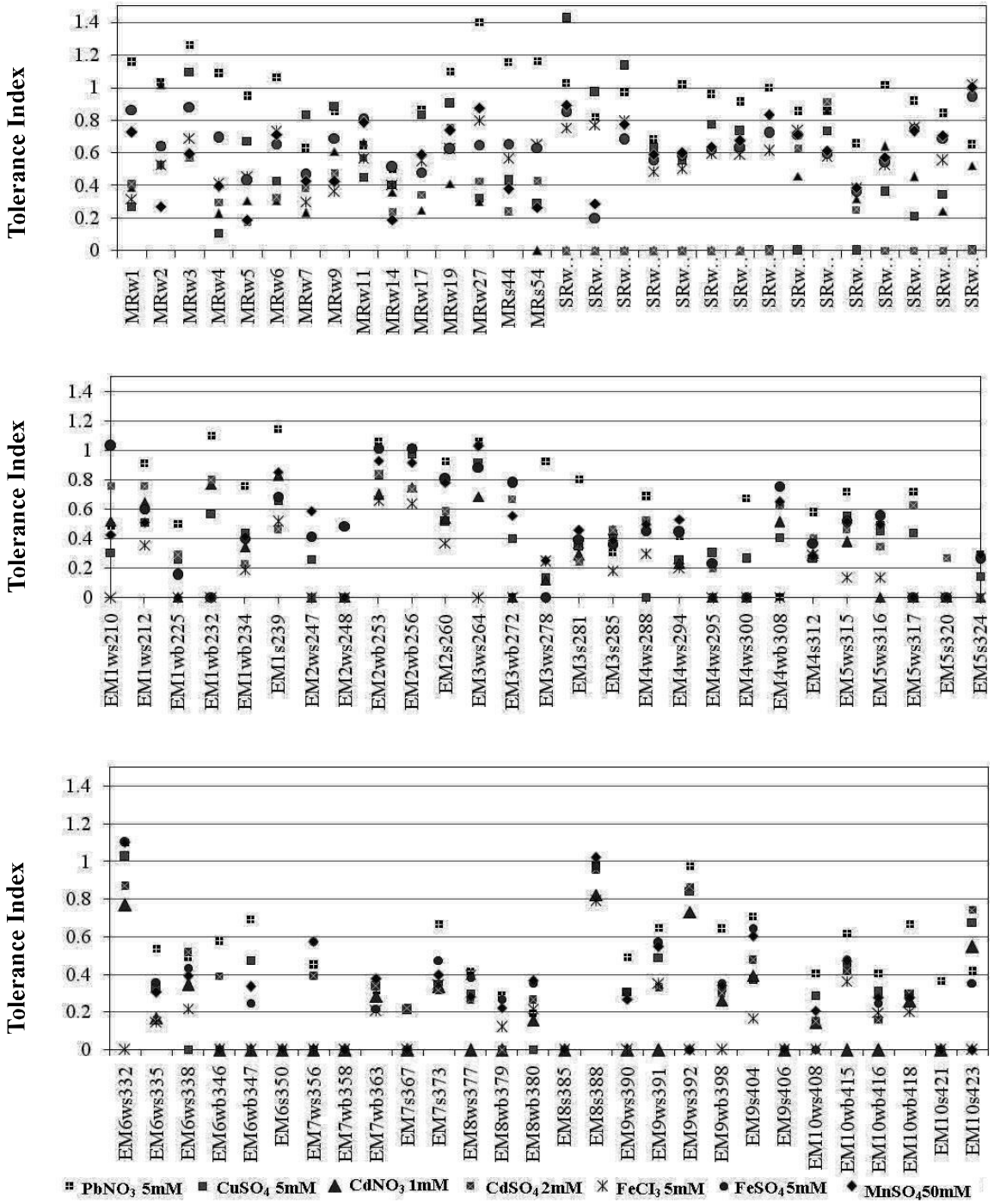


Fig.3.A. Tolerance index of fungal isolates screened for metal tolerance from MR, SR and EM

than cadmium, while in *Aspergillus* and *Fusarium* it was inhibited by all metals. A change in pigment coloration was noted when isolates were grown in presence of iron, becoming orange in *Penicillium* and dark violet to wine red in *Alternaria*.

Metal Sorption

Thirty seven isolates were selected based on high metal MTC values and representative of each genus from different econiches to examine the capacity for metal sorption. Amount of each of the metals sorbed in terms of the sorption coefficient (*Q*) for metal sorbed by the fungal biomass (mg metal g^{-1} dry weight), along with a statistical analysis of their sorption values with respect to the various genera and their species are given in **Table 2.A** and **2.B.** Most of the species of *Penicillium*, *Aspergillus*, *Fusarium* and *Alternaria* showed good sorption ability.

The average range of *Q* values for *Penicillium* species was 26-45 mg Pb^{2+}, 3-6 mg Cu^{2+}, 2-5 mg Cd^{2+} as nitrate, 10-12

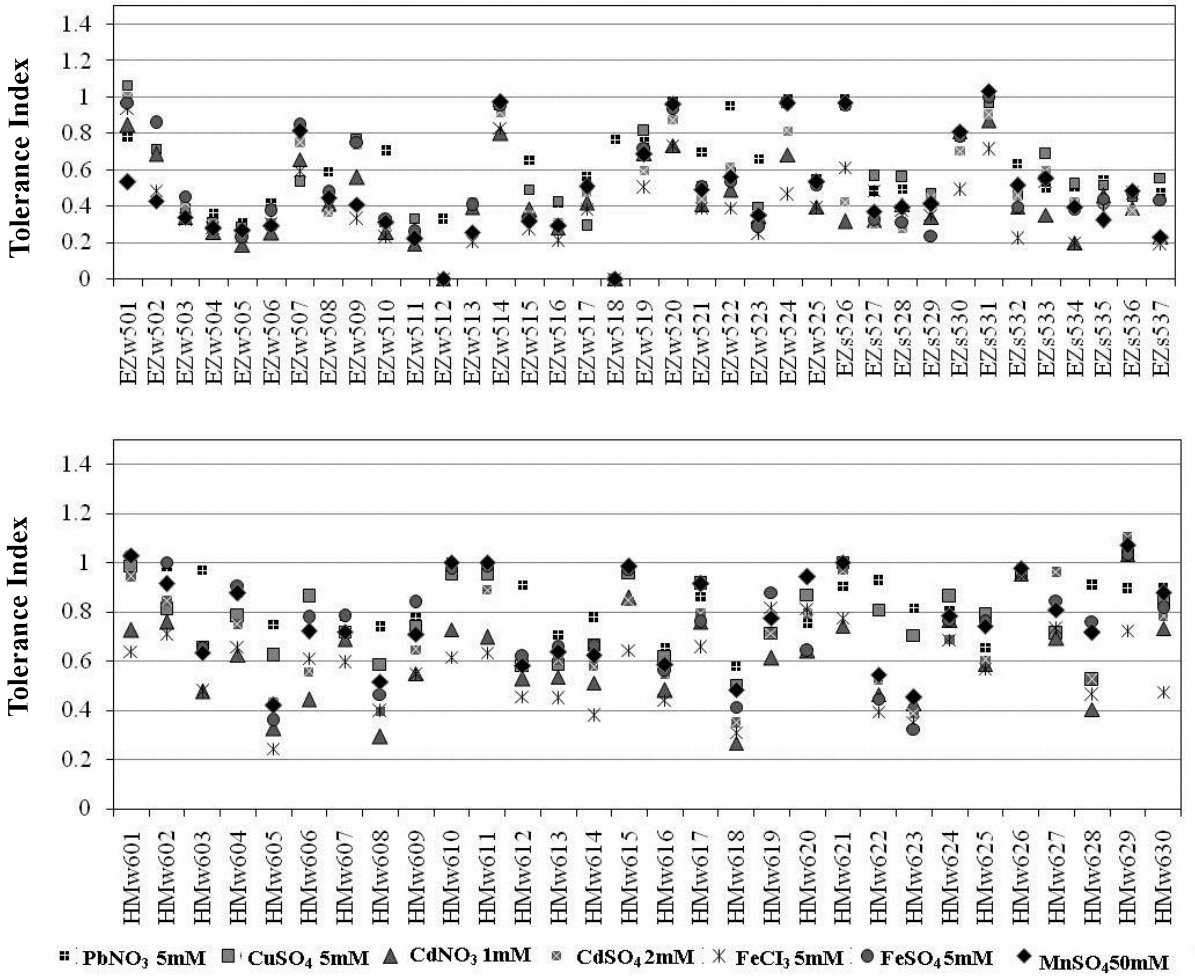


Fig.3.B. Tolerance index of fungal isolates screened for metal tolerance from EZ and HM

mg Fe^{3+}, 16-20 mg Fe^{2+}. In case of Cd^{2+} as sulphate, the isolates from EM, EZ and HM sorbed as much as 25-30 mg Cd^{2+}, while isolates from MR and SR sorbed about 1-5 mg Cd^{2+}. Likewise, isolates from MR and SR sorbed 32-43 mg Mn^{2+} as compared to the isolates from EM, EZ and HM which sorbed an average of 8.5 mg Mn^{2+}. *Penicillium citrinum* Srw119 showed the best sorption capacity, with Q value of 45.22 mg Pb^{2+}, 6.29 mg Cu^{2+}, 12.53 mg Fe^{2+}, 17.57 mg Fe^{2+} and 43.46 mg Mn^{2+} g^{-1} biosorbent.

Similarly, *Q* values for aspergilli were recorded as 20-40 mg Pb^{2+}, 2-4 mg Cu^{2+}, 2-7 mg Cd^{2+} as nitrate, 6-10 mg Fe^{3+}. In case of Cd^{2+} as sulphate, the isolates from EM, EZ and HM sorbed around 32-39 mg Cd^{2+}, with isolates from MR and SR sorbing much less at 1-4 mg Cd^{2+}. Isolates from MR and SR sorbed 18-20 mg Fe^{2+} while those from EM, EZ and HM sorbed only 5-8.5 mg Fe^{2+}. In case of Mn^{2+}, isolates from MR and SR sorbed 32-43 mg Mn^{2+} as compared to the isolates from EM, EZ and HM which sorbed merely 8-10 mg Mn^{2+}.

Q values obtained for *Fusarium* species were 27-37 mg Pb^{2+}, 0.4-0.6 mg Cu^{2+}, 1-6 mg Cd^{2+} as nitrate, 42-46 mg Cd^{2+} as sulphate, 6-9 mg Fe^{3+}, 3-7 mg Fe^{2+} and 10.5 mg Mn^{2+}.

Among the dematiaceous fungi, *Alternaria* species showed a *Q* value of 36-40 mg Pb^{2+}, 5-8 mg Cu^{2+}, 3-6 mg Cd^{2+} as nitrate, 35-37 mg Cd^{2+} as sulphate, 11.5 mg Fe^{3+}, 9-11 mg Fe^{2+} and 10-11 mg Mn^{2+}, while *Cladosporium* species showed 23-33 mg Pb^{2+}, 2-5 mg Cu^{2+}, 5-10 mg Cd^{2+} as nitrate, 35-40 mg Cd^{2+} as sulphate, 6-11 mg Fe^{3+}, 11-16 mg Fe^{2+}. Isolate SRw120 was able to sorb as high as 42 mg Mn^{2+} whereas other isolates could only sorb up to 9-10 mg Mn^{2+}.

There was dissimilarity in heavy metal sorption by the different genera, as well as by the different species within a given genus. However, there was a similarity in sorption of transition metals by the different genera, as well as by the different species within a given genus. In contrast, *Fusarium* and *Alternaria* species yielded similarity in sorption values for both heavy metals and transitional metals, with marginal variations.

DISCUSSION

The levels of heavy metals lead, copper and cadmium, as well as iron, in the Mandovi estuary water samples were found to be within permissible levels, while manganese was present above toxic limits of 0.05 ppm (ATSDR,

2011). The comparatively lower concentration of iron versus manganese could be explained as a result of a greater sedimentation of the iron fines as compared to manganese. This is corroborated by earlier findings that iron in the sediment in these areas, was much higher than manganese (Ratnaprabha and Nayak, 2011) and that almost all of iron but only 7% of manganese gets deposited (Alagarsamy, 2006). The exception in the high iron concentrations at the Mandovi Estuary - Station 3, the Panjim ferry site, could be due to continuous movement of ferries across the estuary as a mode of transport and large recreational yachts, thus slowing down the sedimentation of iron.

The mangrove water sample also registered concentrations below toxic levels of lead and copper; however, cadmium was seen to be well above the permissible limit of 0.1 ppm (ATSDR, 2011). Mangroves serve to entrap not only nutrients, but also pollutants, and their sediments act as a cache (Kathiresan *et al*., 2013); the source of cadmium pollution could be vehicular exhaust from the heavy road traffic adjacent to the mangroves. The contrastingly low level of lead could be due to settling of the heavier lead metal onto the sediment. Alagarsamy (2006) has likewise reported sediments to be primary sinks for lead in aquatic environments. Manganese was also in excess, in keeping with levels of the estuary waters.

Levels of metals in the brine of the salterns were high, as a result of concentration of the water together with the solutes or suspended fines. The source of water to the salterns being from the mangroves, the toxic levels of cadmium and manganese in the mangroves yielded correspondingly higher levels in the brine.

Zuar dockyard water samples contained heavy metals lead and cadmium beyond the toxic limit of 1 ppm for lead and 0.1 ppm for cadmium. The cause of high levels of these heavy metals at the Zuar shipbuilding yard would be from their use as an antifouling agent in paints, the use of lead having been phased out only after 1990s, coupled with ongoing repairs and painting of old barges and consequent scrapings washed into the waters.

The metal contamination at the Mormugao harbor is contributed by the offloading of iron ore, its open air storage and subsequent export. It was observed that the amount of all the metals in the sea water, post monsoon, was doubled as compared to that in pre monsoons, which would be due to the washing of metals into the sea water as a result of rainwater runoffs.

The intense barge movement along the Mandovi and Zuari estuaries, carrying a total of 30 million tons of iron ore per year, or over 82 thousand tons of ore per day at the period of sampling, and the maintenance or building of the barges, would account as the most important factor for the introduction of metals in the estuary.

In this study, the MTC of metals by *Penicillium* and *Aspergillus* species for lead and copper was higher as compared to reported values of 5-12.5 mM Pb^{2+} and 1-5 mM Cu^{2+} (Gopal *et al*., 2002; Al-kadeeb, 2007; Marbaniang and Nazareth, 2007; Zafar *et al*., 2007; Iskandar *et al*., 2011; Gazem and Nazareth, 2013); however, it was less than that of 25 mM Pb^{2+} and 7.5-15 mM Cu^{2+} (Ezzouhri *et al*., 2009). MTC of 2-5 mM cadmium salts by a few *Aspergillus* species were comparable to values reported (Iqbal *et al*., 2006; Zafar *et al*., 2007); that of iron salts at 5-20 mM Fe^{2+} was much higher than earlier reports of 300 mg l^{-1} Fe^{2+} (Osaizua *et al*., 2014).

The isolates showed a higher MTC of lead, than that obtained with the other heavy metals copper and cadmium salts. This corroborates earlier findings with various fungal genera and species (Gopal *et al*., 2002; Taboski *et al*., 2005; Iqbal *et al*., 2006; Zafar *et al*., 2007; Nazareth and Marbaniang, 2008; Leitão, 2009; Nazareth *et al*., 2012). It is possible that the mechanism of tolerance to lead by the isolates involved a cell-surface sorption of metal through a mechanism of ion-exchange, with little or no entry into the cell, thereby reducing the metal toxicity to the cell and increasing the tolerance level, as has been recorded earlier (Gadd, 1994; Volesky and Holan, 1995; Akhtar *et al*., 1996; Kowshik and Nazareth, 1999; Gopal *et al*., 2002; Taboski *et al*., 2005; Akar and Tunali, 2006; Yan and Viraraghavan, 2008; Gazem and Nazareth, 2012; Gazem and Nazareth, 2013). Few isolates showed stimulation of growth at lower concentrations of lead and of copper corroborating earlier reports (Al-kadeeb, 2007; Gazem and Nazareth, 2013).

The tolerance levels of different salts of a given metal also varied: cadmium nitrate was seen to be relatively more toxic than cadmium sulphate. Similarly, the isolates grew at much high concentrations of ferrous salts than that of the ferric salt. Ferric as well as the chloride ions are known to be more toxic than the ferrous sulphate salt (Nissim, 1953; Albretsen, 2006). It has been shown that the type of metal salt can affect the nature of fungal response to the metal (Nazareth and Marbaniang, 2008).

A comparative analysis of metal tolerance levels by the different genera of fungi indicated that isolates of *Penicillium* and *Aspergillus* tolerated higher metal concentrations than those of *Fusarium, Alternaria* and *Cladosporium*, with *Penicillium* being the more tolerant genus. However, *Penicillium* species had a lower tolerance to cadmium nitrate and aspergilli from salterns showed no tolerance to both the cadmium salts. These findings confirm earlier reports (Baldrian and Gabriel, 2002; Iqbal *et al*., 2006; Nazareth *et al*., 2012).

The variation in metal tolerance among species of each of the genera *Penicillium*, *Aspergillus* and *Alternaria* corroborates earlier findings in species of *Penicillium* (Marbaniang and Nazareth, 2007; Leitão, 2009), *Aspergillus* (Akar and Tunali, 2006; Iqbal *et al*., 2006; Zafar *et al*., 2007; Ezzouhri *et al*., 2009; Gazem and Nazareth, 2013), *Piptoporus betulinus* (Baldrian and Gabriel, 2002) and other genera (Gadd, 1994; Nazareth *et al*., 2012). This was in contrast with the similarity seen in metal tolerance among species of the genus *Fusarium* and of *Cladosporium*. However, it was also noted that different species of the genus *Alternaria* from the harbor

site, showed similarity in their tolerance levels, an observation also recorded using *Aspergillus* species (Gazem and Nazareth, 2013).

Higher tolerance to heavy metals by isolates from the Mandovi estuary, Zuar dock and Mormugao harbor over that by isolates from the mangroves and salterns, paralleled the relative differences in levels of metal concentration in these areas. This indicates that the isolates developed an increased tolerance to the metals by constant exposure (Gadd, 1994; Akhtar *et al.*, 1996; Ezzouhri *et al.*, 2009; Banik *et al.*, 2013; Fomina and Gadd, 2014).

The reduction in growth in presence of increasing concentration of metal corroborates earlier reports (Baldrian and Gabriel, 2002); this may be due to the internal accumulation of toxic heavy metals, or probably due to the decrease or inhibition of spore germination possibly caused by the metal entering the spores, associating with the particulate insoluble cytoplasmic component and reacting with cytoplasmic receptor sites (Babich and Stotzky, 1982). The change in growth pattern from a loose nature on control medium without metals to a more dense compact growth with narrower colony diameter in presence of cadmium salts corroborates earlier reports (Nazareth and Marbaniang, 2008). Lead ions showed a lesser effect on the nature of growth of the isolates, in comparison with copper, cadmium, iron and manganese; this has also been observed in isolates of *Penicillium* (Nazareth and Marbaniang, 2008).

The presence of a clear zone around the colony, *Aspergillus* and *Fusarium* species, in response to lead, copper, cadmium and manganese salts, has also been reported for *Aspergillus* species and *Trichoderma asperellum*, as being due to an acidification of the medium caused by active fungal growth (Osaizua *et al*. 2014); this acidification could also be responsible for mineral dissolution (Fomina *et al.*, 2005). This phenomenon was particular to only two of the five genera studied, thus affirming that the response of the isolates to metals was specific to a given genus.

The enhanced pigment production correlating with a greater metal tolerance indicated the role of pigment in protection of the fungus against metal stress, through a possible pigment-metal interaction and consequent lowering of available metal and its toxicity to fungus (Gadd, 1994), in keeping with earlier reports on *Penicillium* (Nazareth and Marbaniang, 2008), *Aspergillus* (Gadd, 1994; Gazem and Nazareth, 2013) and *Fusarium* (Kowshik and Nazareth, 2000).

Metal sorption by the isolates showed that the average range of 20-40 mg g^{-1} Pb^{2+} sorption, be the same as reported values of 22 mg g^{-1} by *Aspergillus versicolor* and *A. flavus*, (Cabuk *et al.*, 2005; Akar and Tunali, 2006) and 30-40 mg g^{-1} for various *Aspergillus* species (Gazem and Nazareth, 2013), although less than the 52-76 mg g^{-1} obtained for some species of *Penicillium* and *Aspergillus* (Iskandar *et al.*, 2011; Iram *et al.*, 2013) and for *Corollospora lacera* (Taboski *et al.*, 2005), it was more than that of 6-10 mg g^{-1} obtained for marine fungi *Monodictys pelagica* (Taboski *et al.*, 2005). The sorption of Fe^{2+} and Mn^{2+} at 6-20 mg g^{-1} and 8-43 mg g^{-1} respectively by the isolates, was much higher than values reported of 1.5 mg g^{-1} for *Aspergillus* species and *Trichoderma asperellum* (Osaizua *et al.*, 2014). The *Fusarium* isolates gave very promising results for sorption of Cd^{2+} as nitrate salt, at 16.78 mg g^{-1}, higher than reported results of 5.5-11 mg g^{-1} of *Trichoderma* species (Mohsenzadeh and Shahrokhi, 2014), or in the case of Cd^{2+} as sulphate salt, a *Q* value as high as 42-46 mg g^{-1}, in comparison to a mere 19 mg g^{-1} of *Rhizopus nigricans* earlier reported (Volesky and Holan, 1995). The dematiaceous fungi, *Alternaria* and *Cladosporium*, also sorbed Cd^{2+} as sulphate to a high degree of 33-40 mg g^{-1} Cd^{2+} hitherto unreported. While most of the reports record cadmium sorption of any one salt, the authors have attempted herein to show the difference in sorption of the cadmium ion, as influenced by the salt composite.

A comparison of sorption between the ferrous and ferric ions showed that *Penicillium* and *Aspergillus* species sorbed ferrous ions to a greater extent than ferric ions, while sorption of both ions by *Fusarium* and the dematiaceous fungi *Alternaria* and *Cladosporium* were near equal.

It was observed that most of the isolates of all the genera obtained could sorb lead significantly, with *Penicillium* and *Aspergillus* also sorbing copper, ferrous, ferric and manganese ions to a considerable degree, but sorption of cadmium was poor. The dematiaceous fungi *Alternaria* and *Cladosporium* also sorbed copper and ferric ions substantially, as well as cadmium as sulphate to a high degree. In contrast, *Fusarium* sorbed cadmium as both nitrate and as sulphate to a large extent, but was less effective in the removal of copper, iron and manganese ions from solution.

Analysis of metal tolerance versus sorption by the various genera showed that, *Aspergillus* and *Penicillium* had high levels of tolerance towards copper ions, however, their sorption capacity was not consistently high, and although *Penicillium citrinum* SRw119 had the highest tolerance to cadmium ions and *Aspergillus fumigatus* $EM2w_b253$ to copper, amongst all the isolates, their sorption capacity for cadmium and copper, respectively was moderate or low. In contrast, those species which had a low tolerance of a given metal could sorb the metal efficiently as was seen in the case of *Fusarium*, and some species of *Alternaria* and *Cladosporium*, in presence of cadmium salts; it was also seen that although a fungal species lacked tolerance to a metal, it was capable of sorbing the same, as exemplified in *Aspergillus* isolates with cadmium salts. This has also been shown in *Rhizopus* and *Aspergillus* isolates (Zafar *et al.*, 2007; Gazem and Nazareth, 2013; Iram *et al.*, 2013). In yet other instances, some species of *Cladosporium* had a low tolerance towards copper, as well as a poor sorption capacity of the metals, while other species such as *Penicillium citrinum* SRw119, possessed a high tolerance to lead, copper, iron and manganese ions, as well as displayed a high efficiency in sorption of these metals. Hence, some isolates had a high maximum metal

tolerance level but low or moderate capacity for metal sorption, while some showed moderate tolerance levels but appreciably good sorption with respect to a given metal; some showed low tolerance limits coupled with a low sorption while others had a high tolerance as well as high sorption capacity. This showed that metal tolerance by a species to a given metal during growth, was independent of its sorption capacity and did not necessarily parallel its sorption of the metal, thus indicating that tolerance and its sorption by a given isolate had no definite correlation, or that metal tolerance is not indicative of the sorptive capacity of a fungus.

The development of resistance by a microorganism to a toxic compound is indicative of the compound or pollutant existing in excess in that environment (Gadd, 1994; Zafar *et al.*, 2007; Rehman *et al.*, 2008; Banik *et al.*, 2013). However, it was observed that the degree of metal tolerance and sorption by the isolates, when compared against metal concentrations in the environment, did not necessarily parallel each other. Observations revealed that while lead levels in the saltern brine, the Zuar docks and the Mormugao harbor were high, the tolerance by the isolates from these econiches was low or moderate, and the sorption was good by isolates from salterns, but low to moderate by those from the docks and harbor. Similarly, copper was high in the Mandovi estuary, Station 5, but copper tolerance by the isolates was low. Cadmium was high in the saltern brine, and while *Penicillium* isolate had a good tolerance and sorption, the aspergilli showed no tolerance, and had a poor sorption capacity for cadmium. This indicated that metal in the environment did not always induce metal tolerance in the fungal organisms, nor did it have a bearing on their sorption capacity, the process of fungal-metal sorption involving functional groups on the cell wall, which are specific to a given genus and/or species (Gadd, 1994; Akhtar *et al.*, 1996; Kapoor *et al.*, 1999; Wang and Chen, 2006; Gazem and Nazareth, 2013).

CONCLUSION

Studies on metal tolerance and sorption by fungi obtained from metal-contaminated econiches were carried out with different genera or species, and with various metals, including different salts of a given metal and/or different valencies. It has been shown that metal tolerance and sorption is affected by the nature of its salt and/or valency. Furthermore, although a genus may have an overall greater capacity for metal tolerance and sorption, as in the case of *Penicillium* and *Aspergillus*, a high tolerance to metals by a fungal species need not correspond to a high metal sorption capacity. Similarly, a fungus having a low growth in presence of metals, may display high sorption ability, due to its inherent characteristic functional groups on its cell wall. Furthermore, fungi existing in a metal-polluted environment did not necessarily develop a high metal tolerance and/or sorption capacity, or may also have been isolated as a chance environmental contamination in the econiches. It therefore appears that the ability of a fungal species for effective interaction with metals is distinctive of a particular species in response to a given metal.

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