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Biodiversity of Fungi from Lead Contaminated Industrial Waste Water and Tolerance of Lead Metal Ion by Dominant Fungi

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ABSTRACT

Lead is important heavy metal found in different parts of the world and its high concentration releases in the environment due to anthropogenic activities. Industries are major sources of emission of lead in the environment. In present study, lead concentration was studied from coal based iron manufacturing industries, iron casting industry and petrochemical industry. The lead concentrations were studied from May, 2010 to April, 2011. All studied industries release lead concentration higher than the permissible limit of standard. Fungi were isolated from industrial effluent containing more than 1.0 mg L^{-1} of lead concentration. Dominant fungi isolated from high concentration of lead were further studied for tolerance against lead nitrate. Total 161 colonies of 37 species were isolated from industrial effluent in which genera of *Aspergillus* were found dominant. Maximum fungi were obtained from Columbia Petrochemical industry. Diversity of fungi is affected according to season and industries. Some fungi were found in all three season and also in high concentration of lead contaminated waste water. The dominant fungi were found tolerant against high concentration of lead nitrate. Hence, these fungi will utilize as biosorbent for absorption of lead from aqueous solution using ecofriendly method.

Key words: Lead pollution, biodiversity of fungi, heavy metals, iron industries, Raipur

INTRODUCTION

The lead concentration in the environment was increased from rapid industrialization during the 18th and 19th centuries (Patterson, 1983). The estimated worldwide anthropogenic emission of lead in the atmosphere includes combustion of coal in power industries, combustion of oil, mining, lead production, steel and iron manufacturing, municipal and sewage sludge, phosphate fertilizers, wood combustion and battery work (Nriagu and Pacyna, 1988). Solid waste of municipal, sewage sludge, industrial wastewater discharge also contains high levels of Pb. Industries are the major source of lead and other metal pollutions because they utilize large amount of raw minerals such as ores of iron, aluminum, bauxite, clay, etc. Toxic metals associated with ores are released from industries to the environment in the form of particulate matter, aerosols, wastewater, solid waste and sludge.

Released effluents from industries becomes a lot of open water which is taken up by nearby plants and enter to animals and mankind through the food chain. Pollution of lead and other heavy metals from industries is burning problem now a day and it was drawn in different part of Australian, African, Asian and Europeans countries (Birch and Taylor, 1999; Nicholson *et al*., 2003; Nadal *et al*., 2004; Al-Khashman, 2004; Azumi and Bichi, 2010; Li *et al*., 2001). Due to rapid urbanization and industrialization, high level of heavy metal has been experienced over different voice of India. Lead and other heavy metals were reported from New Delhi, Lucknow, Varanasi, Bhopal, Pali, Vishakhapattanam, Mumbai, Madurai, Ratnagiri, coast part of Tamilnadu etc. (Banerjee, 2003; Khillare *et al*., 2004; Singh *et al*., 1997; Tripathi, 1994; Shrivastava *et al*., 2003; Sahu and Bhosale, 1991; Krishna and Govil, 2004; Agoramoorthy *et al*., 2008; Sultana and Rao, 1998; Bhaskar *et al*., 2008).

Fungi are structurally unique organisms that contribute to significant removal of metal ions from waste water. This is because of their great tolerance towards metals and other adverse conditions such as low pH and their intracellular metal uptake capacity (Gadd, 1987). Metals and fungi can interact in concert in several ways depending on the type of metals, organism and environment. The impact of heavy metals on the environment has promoted the research to produce alternative, effective and low cost waste water purification scheme. To get rid of the heavy metals from the environment using microbial method, it is necessary to know the tolerance of microbes for particular metal ions. Metal tolerance is defined as the ability of an organism to survive in response to metal. Biological mechanism implicated in fungal survival in metal polluted aqueous solution includes extracellular precipitation, crystallization, transformation of metals, biosorption of cell wall, decreased transport or impermeability, efflux, intracellular compartmentation and sequestration (Gadd, 1993).

Raipur city at latitude 21°24' N and longitude 81°63' E is the capital of Chhattisgarh state India. Recently, World Health Organization (WHO) report concluded in which Raipur has gained third worst polluted city in India and has found its way on the list of top twenty polluted cities in the world (WHO, 2014). Raipur is sandwiched between two largest industrial sites, one by Bhilai steel plant at Bhilai city and second by industrial area of Raipur including, Urla, Siltara, Birgaon, Bhanpuri and Godwara village. Hence, the city picks up pollutants from both sites according to wind direction and water drainage. Raipur is good industrially developed and is an important iron market of India. Many powers, ferrous alloy, steel, cement, rice mill and aluminum industries are working in industrial sites of the city, which are founded on coal burning. The availability of iron ores, coal, bauxite, clay is the chief cause for the formation of these manufactures. The particulate matter of Raipur city has been found to contain high a level of Pb metal ions. The released Pb content in the atmosphere of Raipur, is strongly correlated with the contribution of industries of the city (Sharma and Pervez, 2003, 2004; Patel *et al*., 2008). The burning of coal in industries of the city was found to contribute the lead in the air of Raipur (Patel *et al*., 2010; Thakur *et al*., 2004). The Pb content of Raipur city has likewise found in roadside soils due to heavy traffic load (Kamavisdar *et al*., 2005; Sharma and Pervez, 2003). Due to high Pb content in road side atmosphere the level of lead in blood has been seen high in citizens living nearby highway (Sharma and Pervez, 2003). The soil, sediment, surface and runoff water of industrial area were also found contaminated with Pb metal pollution (Patel *et al*., 2006; Patel *et al*., 2010).

Survey of different pollutants in the environment of Raipur and its surrounding was done and is has found a correlation with industrial activity, motor vehicles of traffic sites and coal combustion. Apart from these studies of heavy metals in industrial effluent is still an untouched area of Raipur and Chhattisgarh. Therefore, the present study proposes to regain out the lead concentration in industries. The data will useful to aware citizens of Raipur and also to design an effective approach towards control of releasing of lead from industries. Fungi isolated at present study from lead contaminated industrial waste water may adopted on it and having potential to resistant high concentration of Pb. So that, the present work not only reporting pollution of lead from industries but asides giving an ecofriendly biological alternate to remove it from aqueous solution.

MATERIALS AND METHODS

Survey sites: The concentration of lead in industrial effluent was studied from coal based iron industries, iron casting industry and a petrochemical industry of Raipur, Chhattisgarh, India. The industries are located in Urla and Birgaon industrial area of Raipur (Table 1). The industrial sites are near about 06 km from the laboratory. Urla and Birgaon are among the most polluted sites of Raipur. These industrial sites include iron based manufacturing industries, rice mills, paper mills, plastic mills, mineral casting industries, power industries, chemical industries, petrochemical industries, dye industries, oil industries, charcoal industries and thus along. Most of the industries have an appropriate drainage system for proper disposal of effluents but few of them releases waste water to the soil surface.

Sample collection and study of lead concentration: Samples were gathered up in sterile air tight plastic bottles. Samples (effluent) were compiled from a drain area of industries generally in the second week of month, in the break of the day between 06-08 am for the period of one year (May, 2010 to April, 2011). After the collection of sample, lead concentration was determined by Spectroquant Nova60 within 06 h of sample collection using PAR methodology.

Isolation of fungi from industrial effluent: After determination of lead concentration, fungi were isolated from industrial samples having more than 01 mg L^{-1} of lead concentration. For isolation of fungi protocol described by Ezzouhri *et al.* (2009) was used with some modifications. Five Petri dishes were used to isolate fungi for different dilution of waste water sample of 10^{-0} (original sample) to 10^{-4} (diluted sample of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4}). After pouring Petri dishes were incubated at 26±1°C for 5-7 days. After incubation period all fungal types were pure culture in PDA slant for further work.

Identification of isolated fungi: Preliminary, identification of fungi was done at the research center (School of Studies in Biotechnology, PT. Ravishankar Shukla University, Raipur) with the help of available literature. The most common fungi were identified by macroscopic examination based on cultural characters observed on PDA medium and microscopic examination by lactophenol cotton blue slide. Further identification of fungi up to species level was done from the National Centre of Fungal Taxonomy, New Delhi. One fungus was identified at the molecular level by sequencing of D1/D2 region of 28S RDNA. The sequenced nucleotides were BLAST to detect the organism. This identification was done from Xcelris laboratories Pvt. Ltd. Ahmedabad.

Table 1: List of industries, where effluents collected

Industries	Types of industry	Locations
Mahindra ispat	Coal based iron industry	Urla industrial area, Raipur
Simplex casting	Iron casting industry	Urla industrial area, Raipur
GK ispat	Coal based iron industry	Urla industrial area, Raipur
GP ispat	Coal based iron industry	Urla industrial area, Raipur
Columbia petrochemicals	Petrochemical industry	Birgaon industrial area, Raipur

Study of tolerance of lead by some fungal species: For lead tolerance five flasks were prepared with one control (without lead nitrate) and four test flasks with different concentration of lead nitrate in 50 mL PDB medium. All concentrations were conducted in triplicate with lead concentration of 50, 100, 500 and 1,000 mg L^{-1} (Modified from Atuanya and Oseghu, 2006). After preparation of flasks, dominant fungi were inoculated in the same amount (1 mL inoculums cultured in PDB medium). Flasks were then incubated to 26±1°C for 7 days. After the incubation period, mycelium were filtered by Whatman filter Paper No. 1 and dried in oven at 80°C for 10 h. Tolerance of lead by fungi was calculated in Tolerance Index (TI) by measuring the Dry Weight (DW) of fungi (Fomina *et al*., 2005).

RESULTS

Concentration of lead from different industrial effluents: The highest concentration of lead was recorded from industrial effluent of Raipur city. The maximum concentration was recorded from summer season followed by winter and rain. Effluent from Columbia petrochemicals showed the highest concentration of lead followed by simplex casting industry (Table 2). Both industries showed lead concentration higher than the allowable limit of national standard in all season. The highest concentration of lead in a particular industry was found 11.58 mg L^{-1} in Columbia petrochemicals, 6.06 mg L^{-1} in simplex casting, 5.01 mg L^{-1} in GK ispat, 2.34 in GP ispat and 1.68 in Mahindra ispat (Fig. 1).

Biodiversity of fungi from different industrial effluent: Fungi were isolated from industrial effluent containing lead concentration more than 1.0 mg L^{-1} (upper permissible limit of national standard). Total 161 colonies were isolated from 37 species. Maximum fungi were isolated from Columbia petrochemicals followed by Simplex Casting industry, GK ispat and GP ispat while least fungi were isolated from Mahindra ispat. Out of 37, 2 species belongs to Zygomycotina, 11 species belongs to Ascomycotina, 24 species belongs to Deuteromycotina (Table 3). Fungi belonging to

Table 2: Concentration of lead in different industrial effluent (mg L^{-1})

	Mean±SE				
Seasons	Mahindra ispat	GK ispat	GP ispat	Simplex casting	Columbia petrochemicals
Rainy	0.19±0.04	0.78±0.22	0.85±0.32	1.42±0.46	3.55±1.85
Winter	0.25±0.06	2.89±0.49	0.94±0.13	2.55±0.93	9.19±0.56
Summer	0.65±0.34	2.73±0.90	1.19±0.40	3.81±0.76	6.44±1.88

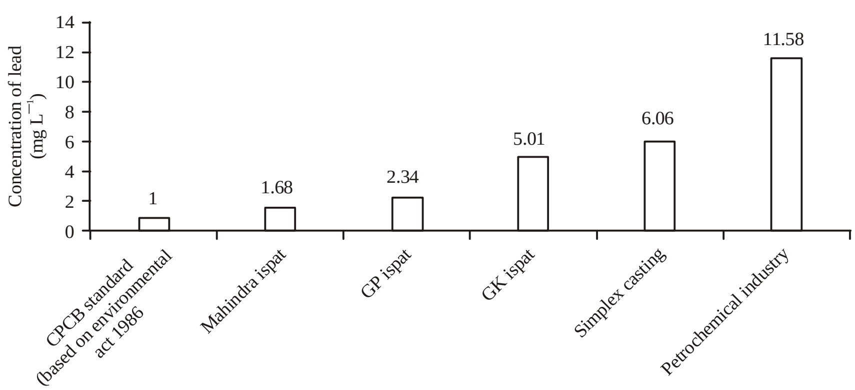

Fig. 1: Comparison of lead concentration between CPCB National standard and highest value recorded in all industrial effluent

Table 3: Industry wise biodiversity of fungi

	F of fungi in different industrial effluent (%)					
Fungi	Mahindra ispat	Simplex casting	GK ispat	GP ispat	Columbia petrochemicals	Total F (%)
Cunninghamella elegans TUFC 20022	00	00.00	0.62	00.00	0.62	1.24
Syncephalastrum racemosum	00	00.00	00.00	0.62	00.00	0.62
Alternaria alternata	00	00.00	00.00	00.00	1.24	1.24
Alternaria sp. 2	00	00.00	00.00	0.62	00.00	0.62
Alternaria sp. 3	00	0.62	00.00	00.00	00.00	0.62
Alternaria sp. 4	0.62	00.00	00.00	00.00	00.00	0.62
Alternaria sp. 5	00	00.00	0.62	00.00	00.00	0.62
Betraniella sp.	00	1.86	0.62	00.00	2.48	4.96
Byssochalamus niveus	00	0.62	00.00	00.00	00.00	0.62
Emericella nidulans	00	00.00	0.62	00.00	00.00	0.62
Eurotium amstelodomi	00	0.62	1.24	0.62	0.62	3.10
Chetomium globosum	00	00.00	00.00	00.00	1.24	1.24
Paecilomyces varioti	00	00.00	00.00	1.86	1.86	3.72
Aspergillus flavus	00	00.00	00.00	00.00	0.62	0.62
Aspergillus flavus var. *scherotorium*	00	0.62	00.00	00.00	0.62	1.24
Aspergillus fumigatus	00	5.59	3.10	3.35	11.18	23.34
Aspergillus niger	00	1.86	1.86	0.62	0.62	4.96
Aspergillus niger var. *scherotorium*	00	1.86	0.62	1.24	1.86	5.58
Aspergillus oryzae	00	00.00	0.62	00.00	0.62	1.24
Aspergillus tamari	00	00.00	00.00	1.24	00.00	1.24
Aspergillus versicolor	00	1.24	0.62	00.00	00.00	1.86
Cladosporium oxysporium	00	4.35	4.35	00.00	0.62	9.32
Cladosporium spherospermum	00	0.62	0.62	00.00	00.00	1.24
Colletotricum sp.	00	0.62	00.00	00.00	00.00	0.62
Curvularia lunata	00	00.00	00.00	00.00	0.62	0.62
Curvularia lunata var. *aeria*	00	00.00	0.62	00.00	00.00	0.62
Curvularia pallescens	00	00.00	00.00	0.62	00.00	0.62
Curvularia verruculosa	00	1.24	00.00	00.00	00.00	1.24
Fusarium clamydosporium	00	6.21	00.00	00.00	00.00	6.21
Fusarium sp. 2	00	00.00	0.62	00.00	0.62	1.24
Penicillium chrysogenum	00	0.62	0.62	1.86	3.10	6.20
Penicillium digitatum	00	2.48	00.00	00.00	00.00	2.48
Penicillium glabarum	00	1.24	00.00	00.00	0.62	1.86
Penicillium oxalicum	00	00.00	00.00	0.62	4.97	5.59
Penicillium purpurogenum	00	00.00	0.62	00.00	00.00	0.62
Phoma chrysenthemicola	00	0.62	00.00	00.00	00.00	0.62
Phoma sp. 2	00	00.00	00.00	00.00	0.62	0.62
Total	0.62	32.89	17.99	13.27	34.75	100.00

Zygomycotina were *Cunninghamella elegans* TUFC 20022 and *Syncephalastrum racemosum*. Fungi of Ascomycotina were *Alternaria alternata, Alternaria* sp. 2, *Alternaria* sp. 3, *Alternaria* sp. 4, *Alternaria* sp. 5, *Betraniella* sp., *Byssochalamus niveus*, *Emericella nidulans*, *Eurotium amstelodomi*, *Chetomium globosum* and *Paecilomyces variotii.*

Fungi belonging to Deuteromycotina were *Aspergillus flavus*, *Aspergillus flavus* var. *scherotorium*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus niger* var. *scherotorium*, *Aspergillus oryzae*, *Aspergillus tamari*, *Aspergillus versicolor*, *Cladosporium oxysporium*, *Cladosporium spherospermum*, *Colletotricum* sp., *Curvularia lunata*, *Curvularia lunata* var. *aeria*, *Curvularia pallescens*, *Curvularia verruculosa*, *Fusarium clamydosporium*, *Fusarium* sp.2, *Penicillium chrysogenum*, *Penicillium digitatum*, *Penicillium glabarum*, *Penicillium oxalicum*, *Penicillium purpurogenum*, *Phoma chrysenthemicola* and *Phoma* sp. 2.

Out of 37 species, 08 species were of *Aspergillus*, 05 species were from the genera of *Penicillium* and 05 species were from the genera of *Alternaria*, 04 species belong to genera *Curvularia*, 02 species isolated from *Fusarium* and 02 species isolated from *Phoma*. Only 01 species were obtained from other genera's. *Aspergillus fumigatus*, *Curvularia lunata* and *Penicillium glabarum* were isolated from industrial effluent containing more than 9.01 mg L^{-1} of lead, while 18.91% fungi were isolated from 7.01-9.00 mg L^{-1} lead (Table 4).

Aspergillus fumigatus, *A. niger* var. *scherotorium* and *Penicillium chrysogenum* were isolated from all three seasons, while *Betraniella* sp., *Eurotium amstelodomi*, *Paecilomyces variotii*, *Aspergillus niger*, *A. versicolor*, *Cladosporium oxysporium*, *Penicillium digitatum* and *P. chrysogenum* were recorded in two different seasons. Fungal species apart from these were isolated in only one season. Highest fungal colonies were held in the month of November 2010 followed by June 2010 and October 2010.

Tolerance of lead by some dominant fungal species: Tolerance of lead by some dominant fungi has been studied by growing fungi in lead containing broth. Dry weight of fungi was compare with control to obtain the tolerance index. The mean dry weight of Aspergillus fumigatus was recorded 0.250 g. Tolerance index of *Aspergillus fumigatus* was found 89.6 in the presence of 50 mg L^{-1} of lead nitrate. The tolerance against the lead by *Aspergillus fumigatus* was decreased to 84.8, 55.2 and 36.8 in the presence of 100, 500 and 1,000 mg L^{-1} lead nitrate, respectively. Dry weight of 0.260 g was obtained in the control biomass of *Fusarium clamydosporium* which was decreased to 0.197, 0.192, 0.067 and 0.058 g of dry weight in supplement of 50, 100, 500 and 1000 mg L^{-1} of lead nitrate. So, the tolerance was decreased with increased concentration of metal ions. Tolerance of 75.00, 55.00, 15.00 and 10.00 has been recorded in supplement of 50, 100, 500 and 1,000 mg L^{-1} of lead nitrate respectively for *Cladosporium oxysporium*. Ninety percent growth of *Cladosporium oxysporium* was inhibited in 1,000 mg L^{-1} concentration of lead nitrate (Table 5 and Fig. 2).

Table 4: Occurrence of fungi from different concentration of lead in industrial wastewater

Concentrations of lead (mg L^{-1})	Occurrence of fungi	F (%)
1.00-3.00	*Alternaria* sp. 2, *Alternaria* sp. 4, *Alternaria* sp. 5, *Aspergillus flavus, Aspergillus oryzae*, *Aspergillus tamari*, *Aspergillus versicolor*, *Byssochalamus niveus*, *Curvularia lunata* var. *aeria*, *Curvularia pallescens, Syncephalastrum racemosum*	31.58
3.01-5.00	*Alternaria* sp. 3*, Aspergillus flavus* var. *scherotorium*, *Aspergillus niger*, *Cladosporium spherospermum*, *Colletotricum* sp., *Emericella nidulans, Eurotium amstelodomi*, *Penicillium oxalicum*, *Penicillium purpurogenum*	24.32
5.01-7.00	*Cunninghamella elegans* TUFC 20022, *Alternaria alternata*, *Curvularia verruculosa*, *Fusarium clamydosporium*, *Penicillium chrysogenum*, *Penicillium digitatum*, *Phoma chrysenthemicola*	18.91
7.01-9.00	*Betraniella* sp., *Chetomium globosum*, *Paecilomyces variotii*, *Aspergillus niger* var. *scherotorium*, *Cladosporium oxysporium*, *Fusarium* sp. 2, *Phoma* sp. 2	18.91
9.01-above	*Aspergillus fumigatus*, *Curvularia lunata*, *Penicillium glabarum*	08.11

Table 5: Tolerance of lead nitrate by some dominant fungi

	Dry weight of fungal mycelium (Average of triplicate) Mean±SE		
Concentration of lead nitrate in medium (mg L^{-1})	AF	FC	CO
Control	0.25±0.01	0.26±0.02	0.20±0.03
50	0.22±0.01	0.19±0.00	0.15±0.01
100	0.21±0.01	0.19±0.00	0.11±0.01
500	0.13±0.00	0.06±0.00	0.03±0.00
1,000	0.09±0.00	0.05±0.00	0.02±0.00

AF: *Aspergillus fumigatus*, FC: *Fusarium clamydosporium*, CO: *Cladosporium oxysporium*

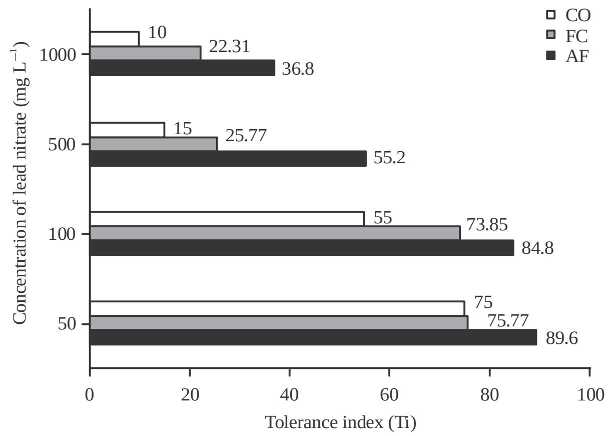

Fig. 2: Tolerance against lead nitrate by some dominant fungi

DISCUSSION

WHO (1995) proposed the collection of lead in different environmental source, including soil, water, air, plants, food should be heightened and the data collected should be made usable for public information and then that masses should make aware and read the lead toxicity and risk linked to soil and aquatic vegetation and fauna. The developing country like India is yet waiting for manufacturing many goods in own country. The establishments of industries for the manufacture of goods are contributing lead and other metal pollution in the industrial area and urban population. The essential and focus of developing country to agriculture, food, manufacture of goods are the only practice, while focusing to control of pollution, cleaning the environment is also pathetic. The advance techniques based on scientific approach for control of heavy metal pollution are not following in industries so the collection of data from industrial pollution is the alone way to aware the people as recommended by the WHO.

Industrial emissions are major sources of lead pollution not only in India but also in different region of the world (Kim *et al*., 1998; Celik *et al*., 2005; Sankpal and Nailwade, 2012; Ladwani *et al*., 2012). Coal combustion is one practice in industries contributed lead pollution in the environment (Patel *et al*., 2006; Pekey, 2006; Patel *et al*., 2010). In present work coal based iron industries were found to contribute lead pollution in Raipur city. Aside from coal combustion in the industries Petrochemical industry was also found a source of lead contamination. Lead and other metal ions pollution from the discharge of petrochemical industry was studied previously also (Nadal *et al*., 2004).

The removal of heavy metals from aqueous solution is another approach toward minimization of heavy metal pollution. The biological method utilizes microbial biomasses for removal of lead and other heavy metals. The industrial effluents are major sources of microbes. The microbial diversity from industrial effluent utilizes for removal and study of tolerance against heavy metals (Siokwu and Anayanwu, 2012; Al-Garni *et al*., 2009; Iram *et al*., 2009). So, present study reports diversity of fungi from high concentration of lead from industrial effluents. Some fungi are found throughout the year, more than one and two seasons. Many fungal species have been considered for adaptation in industrial effluent, municipal sewers, mining sites and many other pollutant sites and these fungi was studied for removal of heavy metals (Iram *et al*., 2009; Ezzouhri *et al*., 2009). The fungi isolated in the present study from high concentration of lead will utilize for removal of lead metal ion from aqueous solution. For this, biomasses of fungi will utilize as biosorbent for removal of heavy metals.

Iram *et al*. (2009) isolated *Aspergillus niger*, *Aspergillus* sp., *Penicillium* sp. and *Fusarium* sp. from industrial and sewage pollutant sources, which found tolerant well against Pb, Zn, Co, Cu and Ni. *Aspergillus niger* and *Penicillium chrysogenum* isolated from municipal sewage by Al-Sohaibani (2011) found to tolerate lead, copper and cadmium at a concentration of 800 mg L^{-1}. Atuanya and Oseghu (2006) isolated *Aspergillus flavus*, *Penicillium* sp. and *Fusarium* sp. from lead contaminated sites were found tolerant to 1,000 mg L^{-1} of lead concentration. Atuanya and Oseghu, 2006) also found that as the concentration of lead increased to 10-1000 mg L^{-1}, decrease in biomass of fungi was recorded. Siham (2007) also recorded effect of lead concentration in biomass of fungi. They isolated *Alternaria clamydospore*, *Aspergillus niger*, *A. oryzae, A. parasiticus*, *A. tamari*, *A. ustus*, *Fusarium* sp., *Penicillium glabarum* from metal contaminated sites which were found tolerant to lead. Decrease in biomass was also recorded by Siham (2007) as the concentration of metal ions was increased.

CONCLUSION

Raipur is industrially developed and many coal based iron industries are working in industrial areas. Lead concentrations of industrial effluent were found several folds higher than the permissible limit of National standard. The citizen living in the industrial area exposes with heavy metals by direct or indirect means. Therefore, control of releasing of heavy metals from industry is a difficult business. Hence, the present study of contamination of lead from industrial effluent helps us to aware the citizens, which will direct approach toward control of lead toxicity to people. The fungi isolated from industrial effluent, which were highly polluted with lead metal ions. The presence of fungi in two or more seasons, indicating its adaptation in polluted effluent. The dominant fungi were found tolerant against high concentration of lead nitrate so these fungi may utilize to remove lead and other heavy metals using as biosorbent.

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