IMPACT: International Journal of Research in Applied, Natural and Social Sciences (IMPACT: IJRANSS)
Vol. 1, Issue 3, Aug 2013, 9-18

ASSESSMENT OF AIRBORNE MICROFLORA IN THE ACADEMIC INSTITUTE OF DEHRADUN

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ABSTRACT

This study was carried out to have an idea of bacterial diversity in our environment so that this could later on serves as a basis for developing database of the microbial load and to have a preliminary idea of the environmental status. An assessment of the air borne bacteria & fungi at Institute campus, Dehradun was experimentally investigated and also checks out their antibiotic sensitivity and heavy metal tolerance activity. Experiments were carried out at 3 different sites (Reception, Toilet, Library) of Dolphin Institute during Months of March, April and May2013. The highest bacterial population was observed in month of March (846×10^{36}) and lowest in the month of May (709×10^{36}).The most dominant bacteria and moulds isolated from air were**:** *Staphylococcus* sp, *Micrococcus* sp, *Bacillus* sp and *Fusarium* sp. Different isolates shows variable antibiotic resistance pattern. For detecting the heavy metal tolerance of bacterial isolates Lead, Copper, Iron and Aluminium at various concentrations (100, 200, 400, 800, 1600 μg/ ml) were used. All isolates shows similar pattern against metals used in the current study i.e. with increase in the concentration of heavy metal the bacterial growth become less with less pigmentation. For the evaluation of tolerance potential among fungal isolates PDA medium was prepared and amended with various concentrations (0, 25 ,50 and 100 μg/ ml) of heavy metal (lead, iron and copper).In case of fungus the zone of diameter decreases with increase in concentration of metals.

KEYWORDS: Antibiotic Sensitivity, Environment, Heavy Metal Tolerance

INTRODUCTION

The impact of various forms of environment pollution is being increasingly felt all over the world (Fasim *et al*., 2003). 90% of world's population lives their life in indoors: in houses, offices, and schools, where they are affected by some indoor environmental factors which influence their health and physical conditions (Stryjakowska-Sekulska *et al*., 2007). There is an emergent need for information about the, distribution and composition of the microbes in atmosphere to support many applications related to public health and so others. Therefore there has been a great interest in indoor microbial studies in recent years (Dong. *et al* 2013, Lou xiuquin *et al*., 2012, Tyagi Shruti *et al*., 2011). The aim of these studies is not only estimation of the air micro flora but also their identification and identifies their antibiotic resistance and heavy metal tolerant activity. Indoor air is mostly affected by bacteria, moulds and yeast. They can be quite dangerous. An epidemiological study says that high concentration of airmicroflora can be allergenic and quite lethal (Stryjakowska *et al*., 2007). Bacteria can combat with heavy metal and antibiotic in environment, they also have the ability to detoxify the effect of these pollutants. Bacterial strains showing multiple resistances to antibiotics and heavy metals have been isolated from many parts of the world. Presence of bacteria showing high resistance to heavy metal or antibiotics is an indicator of

pollutants in the environment where these bacteria are surviving. Metal resistance and antibiotic resistance genes are often carried on the same plasmid. Bacteria are often used as indicators of environmental pollution.

Heavy metals have their carcinogenic and mutagenic nature, so they can cause a quite lethal effect on indoor environment. Their high concentration in air may cause some serious problems like respiratory lung problems, skin problems irritation of eyes, dryness of mucous membrane, headache, dizziness etc. Isolated species of airmicroflora from polluted site have quite excellent property to remove that significant amount of heavy metal from that site (Gavrilesca, 2004; Baldrian, 2003; Zafar *et al*., 2007). Fungi are the dominant organism in some polluted sites, where they show highly tolerant activity against heavy metals. A dark color pigment present in fungi, helps him to reduce the toxic effect of heavy metals.

The aim of this work is observation of microbiological quality of indoor air in selected sites of institute's building of DIBNS located in the Dehradun, where thousands of people work and study

MATERIAL AND METHODS

Trapping Sites

To find out the numbers and types of air borne bacteria and fungi, three different sites (Reception, Toilet, and Library) of Dolphin Institute were selected.

Sampling Procedures

The study was carried out from March 2013 to May 2013.The cultural plate exposure method was adopted for trapping the air borne micro flora (Fasim *et al*., 2003). Sampling was carried out at regular interval of 15 days. The exposed plates containing the growth medium were allowed to stay for 10 minutes of exposure. The time of sampling was kept uniform at all the sites between 10 am to 12 am. After exposure, the plates were transported in a clean container to the laboratory for microbiological examination.

Detection of Colony Forming Unit (CFU/m^3)

The nutrient agar, blood agar and glucose yeast extract agar plates were incubated at 37°C for 24 hours while the sabouraud dextrose agar plates at 28 °C for 2 to 3 days. Bacterial colonies that developed on the plates were isolated and subcultured in fresh nutrient agar slants while the fungi were subcultured in sabouraud dextrose agar. The total number of colony forming unit (cfu) was enumerated and converted to organisms per cubic meter air according to the following equation (Stryjakowska-Sekulska *et al*., 2007).

$$CFU/m^3 = a \cdot 10000/p \cdot t \cdot 0.2$$

Where:

a – the number of colonies on the Petri plate

p – The surface of the Petri plate

t – The time of Petri plate exposure

Identification of Isolates

Identification of Bacteria

The bacterial cultures were identified on the macroscopic (shape, size, color, margin, elevation, opacity consistency, appearance of colony and hemolytic reactions) and microscopic (grams staining and endospore

staining).examinations .Biochemical characterization of recovered isolates were performed according to Bergey's Manual of Determinative Bacteriology. Further final identification was done using **ABIS** software.

Identification of Fungi

The fungal cultures were identified on the basis of macroscopic (colonial morphology, color, texture, shape, diameter appearance of colony and basis of lacto phenol staining) and microscopic (septation in mycelium, presence of specific reproductive structures, shape and structure of conidia, and presence of sterile mycelium) characteristics (Zafar *et al*., 2007).

Relative microbial distribution was conducted according to (Smith *et al*, 1960) where

$$\textit{Relative distribution} = \frac{\text{Number of colonies of the genus or species}}{\text{Total number of colonies of all genera or specie}} \times 100.$$

Antibiotic Resistance Test

All the isolates were tested for antibiotic sensitively by Kirby- Bauer disc diffusion method (Bauer *et al*; 1966). The following antibiotics were used for bacteria: Chloramphenicol (30mcg), Tetracycline (15 mcg), Ampicillin (10mcg), Amikacin (30mcg) and Gentamycin (10 mcg). For fungi: Amphotericin B (50mcg), Clotrimazole (10mcg), and Nystatin (50mcg).

Heavy Metal Tolerance Test

Bacteria

Pure culture isolated from all the sampling was inoculated in the Nutrient broth and the turbidity was matched with the Mc Farland standard. Basal media Nutrient agar (NA) with the heavy metal Lead (Pb) Copper (Cu), Iron (Fe) and Aluminum (Al) were prepared separately. The concentration of heavy metal was maintained as follows: 100, 200, 400, 800, 1600 μg/ ml of Lead (Pb) Copper (Cu), Iron (Fe) and Aluminum (Al)

Fungi

For the evaluation of tolerance potential among isolated fungal strains, PDA medium was prepared and amended with various concentrations (0, 25,50 and 100 μg/ ml) of heavy metal (lead, iron and copper).Media was autoclaved for 20 min at 121 °C and poured into Petri plates. The plates were incubated at 28 °C for 3-7 days. The growth of fungi was monitored from the point of inoculation or centre of the colony. Tolerance was measured by observing the tolerance index (Tahir Arifa., 2012).

$$\text{T.I.} = \frac{\text{Radial growth rate in metal treatment}}{\text{Radial growth rate in control}}$$

RESULTS

The Total Number of Organisms Recovered (CFU/m^3) from Different Sites

In the present study total 72 plates were exposed during three months in three different locations of Dolphin institute of biomedical and natural science, Dehradun. A total of 68 bacterial and 29 fungal isolates were obtained. These isolates were purified and checked for their ability to resist to different antibiotics and heavy metals. The highest percentage contribution of bacterial population was exhibited at Reception, where the air was constantly being stirred by different micro-organisms. Isolation of bacteria and fungus was done after an interval of fifteen days at all three sites, as

per the sampling from March 2013 to May 2013. The CFU/m3 was maximum on March i.e. 846×10^{36} and minimum in May i.e. 709×10^{36} including all the three considered sites. According to different media used in above work, the maximum and minimum growth was observed on Blood agar 506×10^{12} and GYA agar 109×10^{12} respectively (**Table:1**) Site wise bacterial population states in **Figure 1** that the similar bacterial population occurred on Reception (926×10^{36}) and Toilet sites (926×10^{36}).

Table 1: Total Population Count (CFU/m^3) at Different Months from Various Sites

Sites	Media	March		April		May		Total
		1st	2nd	3rd	4th	5th	6th	
Site-1	NA	87×10^{2}	43×10^{2}	6×10^{2}	56×10^{2}	6×10^{2}	4×10^{2}	202×10^{12}
	Blood	81×10^{2}	138×10^{2}	43×10^{2}	116×10^{2}	59×10^{2}	69×10^{2}	506×10^{12}
	GYA	10×10^{2}	69×10^{2}	37×10^{2}	56×10^{2}	43×10^{2}	3×10^{2}	218×10^{12}
Site-2	NA	7×10^{2}	69×10^{2}	43×10^{2}	75×10^{2}	100×10^{2}	7×10^{2}	301×10^{12}
	Blood	119×10^{2}	87×10^{2}	$10\times*10^{2}$	141×10^{2}	81×10^{2}	9×10^{2}	447×10^{12}
	GYA	4×10^{2}	53×10^{2}	34×10^{2}	40×10^{2}	43×10^{2}	4×10^{2}	178×10^{12}
Site-3	NA	43×10^{2}	06×10^{2}	43×10^{2}	4×10^{2}	50×10^{2}	81×10^{2}	227×10^{12}
	Blood	12×10^{2}	7×10^{2}	4×10^{2}	50×10^{2}	87×10^{2}	9×10^{2}	169×10^{12}
	GYA	7×10^{2}	4×10^{2}	4×10^{2}	40×10^{2}	4×10^{2}	50×10^{2}	109×10^{12}
Total		**370×10^{18}**	**476×10^{18}**	**224×10^{18}**	**578×10^{18}**	**473×10^{18}**	**236×10^{18}**	**1431×10^{108}**

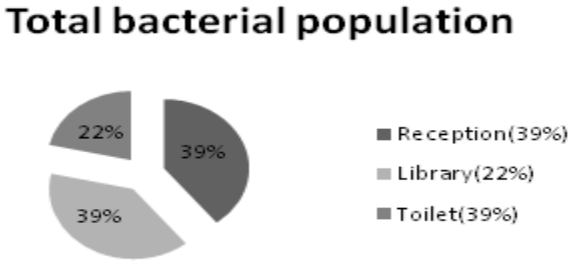

Figure 1: Prevalence of Bacteria from Different Sites

Relative Frequency of Recovered Isolates

Relative frequency was calculated to find out the percentage of recovered isolated bacteria and fungi. The highest percent of isolated bacteria was *S. aureus* (48.52) and then followed *by Coagulase –ve staphylococcus (26.47), A.fecalis* (8.82), *Bacillus cereus* (6.66), *Micrococcus lutes (*4.41), *Bacillus species (3.33*), *Bacillus megaterium* (1.47) and *E. coli* (1.47) **(Table: 2)**. Most dominant fungus was *Fusarium* (34.48) and then followed by *Alternaria* (31.57), *Aspergillus sp* (24.13) and *Cladosporium* **(**20.68) **(Table: 3)**.

Table 2: Monthly Contributions of Recovered Bacterial Isolates

Isolated Bacteria	6 March	21 March	5 April	21 April	4 May	19 May	Total
	1st	2nd	3rd	4th	5th	6th	
A.fecalis	-	02	03	01	-	-	06
Bacillus cereus	-	-	-	01	01	02	04
Bacillus species	02	-	-	-	-	-	02
Bacillus megaterium	-	-	-	01	-	-	01
Coagulase –ve staphylococcus	04	01	02	03	04	04	18
E. coli	01	-	-	-	-	-	01
Micrococcus lutes	-	03	-	-	-	-	03
S. aureus	06	06	04	05	07	05	33
Total	**13**	**12**	**09**	**11**	**12**	**11**	**68**

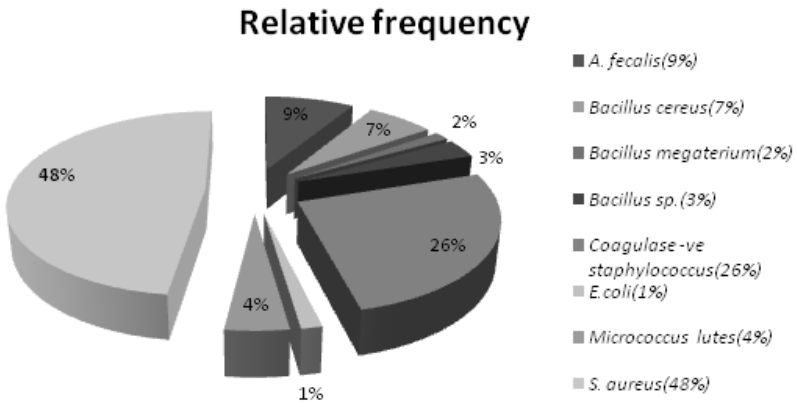

Figure 2: Relative Frequency of Bacterial Isolates

Table 3: Monthly Contribution of Recovered Fungal Isolates

Isolated Fungus	6 March	21 March	5 April	21 April	4 May	19 May	Total
Alternaria	02	02	-	-	-	01	06
Aspergillus species	02	-	01	-	02	02	07
Cladosporium	01	02	01	01	01	-	06
Fusarium	01	-	02	04	02	01	10
Total	**06**	**04**	**04**	**06**	**05**	**04**	**29**

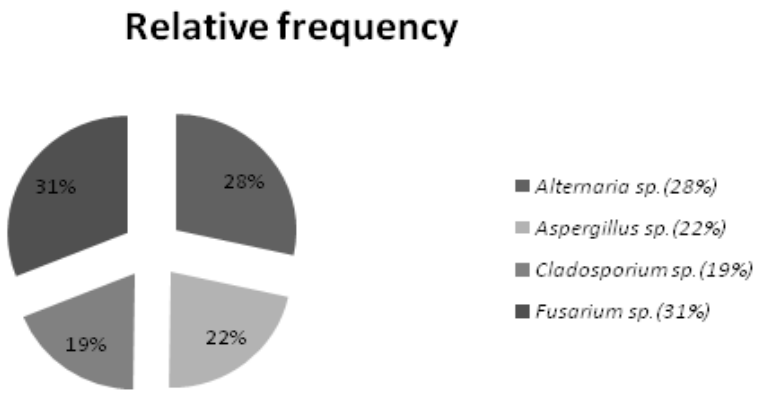

Figure 3: Percentage Contribution of Recovered Fungal Isolates

Antibiotic Resistance Test

Antibiotic resistance test was done against all the bacterial and fungal isolates to check their sensitivity or resistance against selected antibiotics. For antibiotic resistant activity of bacteria the *Ampicillin* antibiotic is commonly resistant for all isolates, except *Bacillus cereus* and *Bacillus megaterium (***Table: 4***).*There is only one antibiotic shown resistance against *Fusarium* sp. and all other antibiotics are sensitive for other fungal isolates (**Table: 5**).

Table 4: Antibiotic Resistance Pattern of Bacterial Isolates

Isolates	AMP^{10}	AK^{30}	GEN^{10}	TE^{30}	C^{30}
A.fecalis	Resistance	22.6 (S)	27.6 (S)	25.6(S)	31.6(S)
Bacillus species	Resistance	21.3 (S)	20.3 (S)	22.6 (S)	25.3 (S)
Bacillus cereus	23.0(S)	20.3(S)	22.3(S)	25.3(S)	26.0(S)
Bacillus megaterium	27.6(S)	25.3(S)	25.6(S)	22.3(S)	28.3(S)
Coagulase –ve Staphylococcus	Resistance	24.6(S)	19.0(S)	25.3(S)	30.6(S)
E. coli	Resistance	21.6 (S)	29.0(S)	22.6(S)	25.6(S)
Micrococcus lutes	Resistance	22.6 (S**)**	24.0 (S)	19.6 (S)	28.3(S)
S. aureus	Resistance	23.0(S)	20.3(S)	22.3(S)	25.3(S)

AMP$^{10=}$ Ampicillin (10mcg)

AK$^{30=}$Amikacin (30mcg)

GEN$^{10=}$ Gentamycin (10 mcg)

TE$^{30=}$Tetracycline (30mcg)

C$^{30=}$Chloramphenicol (30mcg).

Table 5: Antibiotic Resistance Patterns of Fungal Isolates

Isolates	AP^{50}	CC^{10}	NS^{50}
Aspergillus.sp	14.6(S)	14.0(S)	16.0(S)
Fusarium sp.	(R)	21. (S)	15.0 (S)
Alternaria	23.0 (S)	22.5(R)	21.6(S)
Cladosporium	24.6(S)	31.5(S)	32.4(S)

AP$^{50=}$ Amphotericin- B (50mcg)

CC$^{10=}$ Clotrimazole (10mcg)

NS$^{50=}$ Nystatin (50mcg)

Response to Heavy Metals

Heavy metal tolerance was checked against all the recovered bacterial and fungal isolates.

Heavy Metal Tolerance of Bacteria

Tolerance against all bacterial isolates shows similar pattern against all metals (PbcooH, FeCl2, AlCl2,Cuso4) used in the current study i.e. with increase in concentration of heavy metal the bacterial growth become less with less pigmentation. At the concentration of 100, 200, 400 µg/ ml growth was comparable to control. The concentration of 800 there was growth but without pigmentation. at 1600µg/ ml bacterial isolates didn't showed any growth as during preparation of media with copper sulphate and Iron chloride media didn't got solidified. (**Table 6**)

Table 6: Heavy Metal Tolerance Pattern of Bacterial Isolates

Heavy Metal	Concentration	*A.Fecalis*	*Bacillus cereus*	*Bacillus megaterium*	*Bacillus Sp.*	*Coagulase–ve Staphylococcus*	*E. coli*	*Micrococcus luteus*	*S. aureus*
PbcooH	100	+++	+++	+++	+++	+++	+++	+++	+++
	200	+++	+++	+++	+++	+++	+++	+++	+++
	400	++	++	++	++	++	++	++	++
	800	+	+	+	+	+	+	+	+
	1600	-	-	-	-	-	-	-	-
$FeCl_2$	100	+++	+++	+++	+++	+++	+++	+++	+++
	200	+++	+++	+++	+++	+++	+++	+++	+++
	400	++	++	++	++	++	++	++	++
	800	+	+	+	+	+	+	+	+
	1600	-	-	-	-	-	-	-	-
$CuSo_4$	100	+++	+++	+++	+++	+++	+++	+++	+++
	200	+++	+++	+++	+++	+++	+++	+++	+++
	400	++	++	++	++	++	++	++	++
	800	+	+	+	+	+	+	+	+
	1600	-	-	-	-	-	-	-	-
$AlCl_2$	100	+++	+++	+++	+++	+++	+++	+++	+++
	200	+++	+++	+++	+++	+++	+++	+++	+++
	400	++	++	++	++	++	++	++	++
	800	+	+	+	+	+	+	+	+
	1600	-	-	-	-	-	-	-	-

+++ = Growth with pigmentation
++ = growth with less pigmentation
+ = Growth but no pigmentation
- = No growth

Heavy Metal Tolerance of Fungus

In fungal isolates the zone of diameter decreases with increase in concentration of metals. Maximum tolerance index was observed against iron chloride at the concentration of 25µg/ml by *Fusarium* that was 2.0 and the minimum tolerance index was observed against lead acetate at the concentration of 100µg/ml by all the fungal isolates, no growth occurred at this concentration(**Table: 7**).

Table 7: Tolerance Index of Fungal Isolates against Different Heavy Metals

Isolated fungus	$PbcooH$							$FeCl_2$							$CuSo_4$							
	100	TI	50	TI	25	TI	0	100	TI	50	TI	25	TI	0	100	TI	50	TI	25	TI	0
Alternaria sp.	0	0	2.9	1.4	2.6	1.3	2	1.7	0.8	1.8	0.9	2	1	2	0.9	0.4	1.4	0.7	2	1.0	1.9
Aspergillus sp.	0	0	1.2	0.4	1.3	0.4	3	1.3	0.4	1.3	0.4	1.4	0.4	3	1	0.3	1.2	0.4	1.5	0.5	3
Cladosporium sp.	0	0	2.4	1.2	1.2	0.6	2	3	1.5	2.6	1.3	3.4	1.7	2	2	1	2.6	1.3	2.7	1.3	2
Fusarium sp.	0	0	2.7	1.8	1.4	0.9	1.5	2.8	1.8	2.9	1.9	3.1	2	1.5	1.6	1	2	1.3	2	1.3	1.5

DISCUSSIONS

The present study demonstrated that the concentration of culturable bacteria and fungi in the DIBNS campus, Dehradun from 3 sampling sites. Among a total of 68 bacterial isolates from the sampling sites, the number and concentration of airborne Gram-positive bacteria were significantly higher than that of airborne Gram-negative bacteria in indoor environments in DIBNS campus. The explanation of this conflict phenomenon was that Gram-positive bacteria in the air had greater resistance and survival ability than Gram-negative bacteria under strong sunlight (Xie *et al*., 1988). The most common bacteria groups in reception, toilet, and library in the DIBNS campus were *S.aureus, Coagulase –ve Staphylococcus, A.fecalis, Micrococcus lutes, Bacillus species, Bacillus cereus, Bacillus megaterium,* and *E.coli* according to priority, some of which had been reported as the most prevalent airborne bacteria in indoor environments in other studies, such as elementary school (Liu *et al*., 2000), crowded and underground public concourse (Seino *et al*., 2005), and university hospital (Sarica *et al.*, 2002), child day care center (Aydogdu *et al*., 2010), feedstuff-manufacturing factories (Kim *et al*., 2009). Additionally, the most common bacteria in indoor air in the campus were consistent with our former findings carried out in indoor environments in Beijing, while some differences were also observed in the order of most common bacteria (Fang *et al*., 2007).

Same as in bacteria, fungi also have variation in relative frequency value. In this the most dominant fungus is *Fusarium* followed by *Aspergillus* sp, *Alternaria* and *Cladosporium.*

Here we have total 68 bacterial isolates, which are tested against five antibiotics (Ampicillin (10mcg), Amikacin (30mcg), Gentamycin (10 mcg), Tetracycline (30mcg), Chloramphenicol (30mcg)). Among these isolates only two bacterial strains show a little bit different results, in which *Bacillus cereus* and *Bacillus megaterium* are sensitive against Ampicillin. The majority of the bacteria such as *Micrococcus lutes, A. fecalis, Bacillus sp, Coagulase –ve Staphylococcus, E.coli a*nd *S. aureus* were found to be resistant to Ampicillin. Same work was done by some researchers (Karen Michael, 2011, Tee and Najiah., 2011)

There was a possibility of the atmospheric bacteria becoming antibiotic resistant and in some cases antibiotic resistance was present with metal resistance gene, in this way metal resistance could have also been transferred to atmospheric bacteria. In our study program four fungal specimens were test against three antibiotics (Amphotericin- B

(50mcg), Clotrimazole (10mcg), and Nystatin (50mcg)). Where we have minimum sensitivity shown by Aspergillus sp. For Amphotericin antibiotic, and there is also Resistency shown by Fusarium sp against Amphotericin antibiotic.

All bacterial Isolates were tested against different concentrations of heavy metals (CuSo4, FeCl2, AlCl2, PbcooH), ranging from 100, 200, 400, 800 and 1600µg/l. In our study, heavy metal resistance varies as in the pattern of similar results for all isolates. As the concentration of heavy metal increases, the microbial growth of isolates reduced.

At the concentration of 1600 µg/l of all heavy metals in growth media is heavily toxic. At this concentration the bacteria couldn't survive and show no growth and the media also didn't get solidified.

In the present study, the tolerance potential of various isolated fungi from DIBNS campus like *Aspergillus sp.*, *Alternaria*, *Cladosporium* and *Fusarium* were investigated against heavy metals (Cu, Fe and Pb).

A range of fungi from all major taxonomic groups may be found in metal-polluted habitats and the ability to survive and grow in the presence of potentially toxic concentrations is frequently encountered (Ross, 1975; Gadd, Edwards, 1986).

The results of the present study demonstrated that different species of fungus show different tolerance pattern. Their behaviors against different metals are quite different and measured on the basis of Tolerance index. The variation in the metal tolerance may be due to the presence of one or more strategies of tolerance or resistance mechanisms exhibited by fungi. It must also be taken into account that the contamination at the polluted sites is usually not caused by a single metal and that the selection is probably driven either by the most toxic element or by more different metals acting synergistically (Baldrian and Gabriel, 2002). All fungal strains exhibited growth at lower concentration of metals but it became reduced in the presence of higher concentration. In our study the highest tolerance index is shown by Fusarium at the concentration of 25µ gm/L are 2.0.

As the concentration on heavy metals increases the value of tolerance index decreases. The same increment and reduction in growth was noted during the study on filamentous fungi belonging to the genera *Aspergillus*, were more resistant to Cr at higher metal concentrations and suddenly the growth pattern changed (Valix *et al*., 2000). Species of fungi of the genera *Fusarium* and others have been isolated from contaminated soils, and their ability to tolerate the presence of different heavy metals has been analysed by Zafar *et al*. (2007). Ezzouhri *et al*. (2009) also screened fungi (*Fusarium* sp.) for their resistance to heavy metals.

The results revealed that the majority of the isolates were resistant to Fe and Cu, but in case of Pb their tolerant activity is quite low in compare of other metals. On the concentration of 100µ/l their no growth shown by isolates. The level of resistance depends on the isolate tested, as well as the site of its isolation. However, some authors found that microorganisms isolated from contaminated sites were more tolerant than those from natural environments (Massaccesi *et al*., 2002; Malik, 2004).

CONCLUSIONS

- The highest bacterial population was observed in month of March 13(846×10^{36}cfu/m^3) and lowest in May13 (709×10^{36} cfu/m^3).
- The highest relative frequency was found in *S. aureus* i.e. 48.52. and lowest in *bacillus megaterium* and *E coli* i.e 1.42.
- The most dominant isolated fungus was *Fusarium* (34.48) and the least was *Cladosporium* (20.68).

- Antibiotic resistance pattern shows variable results in terms of both bacteria and antibiotics. Ampicillin (10mcg) found Resistant against most of the isolates except *Bacillus cereus* and *Bacillus megaterium* .Amikacin (30mcg), Gentamycin (10mcg), Tetracycline (30mcg) and Chloramphenicol (30mcg) were sensitive for all isolates.
- In heavy metal tolerance, all the bacteria shows similar pattern at 100, 200, 400$\mu g/ml$ concentration bacteria showed growth and pigmentation as well ,at the 800$\mu g/ml$ there was less growth with less pigmentation and at 1600$\mu g/ml$ there was no growth.
- Heavy metal tolerance against fungus, maximum tolerance was occurred in *Fusarium* sp. at 25µg/ml on iron chloride and the minimum tolerance was observed against lead acetate by *Aspergillus* sp at similar cncentration .

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