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Identification and Characterization of Microbes from Industrial area for their Heavy metal Tolerance against Cadmium, Lead and Mercury

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Abstract

The search for bacteria and fungus capable against metal toxicity starts with their isolation from the waste water released from the industries that uses either the heavy metals in their process or releases heavy metals as their waste product. The waste effluent released from paper, textile, paint and iron processing industries were collected and different microbial colonies were isolated from those waste water by standard plating methods, identified by their colony morphology, staining methods and different biochemical procedure. Those isolates were then screened for their antibiotics sensitivity and heavy metal toxicity test. From the antibiotics sensitivity test, Erythromycin and Streptomycin proved to be better antibiotics against isolated bacteria and Tetracycline and Ampicillin proved to be better against fungal isolates. Those antibiotics can be used as good selection markers in the molecular biology techniques. For heavy metal toxicity test, three heavy metals such as Cadmium, Mercury and Lead were analyzed at different concentrations such as 1mM, 5mM, 10mM and 20mM for up to 72 hours for bacterial isolates and 144 hours for fungal isolates. The potential isolates were selected over their growth rate at higher concentration of heavy metals. Bacterial isolates such as Bacillus megaterium, Bacillus licheniformis, Pseudomonas fluoroscence, Pseudomonas syringae, Bacillus subtilis, Corynebacterium xerosis, Bacillus macerans and fungal isolates such as Fusarium, Aspergillus niger, Aspergillus flavus, Cladosporium proved to be the better isolates that can be exploited at their molecular level for the bioremediation of heavy metal contamination.

Keywords: Biochemical methods, antibiotics sensitivity test, heavy metal toxicity test.

Introduction

The influx of heavy metals into waste is mainly due to the intended use of heavy metals in industrial products. At the end, these either will end up in waste to the extent they are not attractive for recycling. Heavy metals may also channel to waste during production and utilization phases. The loss in the manufacturing process is often disposed of as manufacturing waste, while products may be exposed to wear and tear and inclusive corrosion during the use phase. Ongoing research and development in the different processes such as speciation of metals, their toxicity, bioaccumulation, biomagnification, bioindication, migration, removal, biomonitoring must be conducted that enable optimal usage, reusability and bioremediation of these heavy metals[1]. The new methodology of using microbial cultures other than phytoremedial procedures for bioremediation proved to be a good alternative to chemical and other conventional methods of reducing the heavy metal contamination in the soil and water in-effect to rapid industrialization process. The microbial bioremediation is simple, cost effective, safe and comparatively a faster process[2, 3, 4].

Methodology

Water sample collection: The waste water sample from the four sampling points such as sample i. From near paper industries, sample ii. From near textile industries, sample iii. From near paint industries and sample iv. From iron processing industries were collected in sampling bottles.

Isolation and Identification of micro-organisms: The water sample was collected from different sites near to that of industries were first serial diluted, 100µl of the diluents from the samples were taken and then they were spread plated on Nutrient Agar Media[5], and Sabouraud Dextrose agar (containing 0.5% Chloramphenicol antibiotics)[6]. The Nutrient Agar Medium was incubated in incubator at 37^0C for 24 hours. The Sabouraud Dextrose agar plates were incubated at 30^0C for 96 hours. The organisms isolated from Nutrient Agar Media were first screened by their colony morphology and then were gram stained[7] to identify their structure. Then they were identified by different biochemical test as suggested in *Bergey's Manual of Determinative Bacteriology*, 9th Edition[8]. The unknown bacteria were identified by different staining methods and biochemical tests[9-12]. The fungus were identified as in James, G. C. and Natalie, S.[13].

Antibiotics sensitivity test for bacteria: The antimicrobial activity of different antibiotics was determined in accordance with agar-well diffusion method as described by Rious et al.[14].

The bacterial and fungal isolated were first grown in Nutrient broth and Sabouraud dextrose broth (containing 0.5% Chloramphenicol antibiotics) respectively and standardized to 0.5 McFarland standards (106 cfu/mL). 200µL of standardized cell suspension of bacterial and fungal isolates were spread over Mueller-Hinton agar and Potato Dextrose agar (containing 0.1% Streptomycin antibiotics) respectively. Wells were then bored into the agar using a sterile 6mm diameter cork borer. Then 100µL of standardized solutions of Ampicillin (5000µg/ml), Chloromphenicol (5000µg/ml), Tetracyclin (5000µg/ml), Kanamycin (5000µg/ml), Erythromycin (5000µg/ml), Streptomycin (5000µg/ml) and Nalidixic acid (5000µg/ml) were pipette into the wells. The bacterial plates were incubated at 37ºC for 36 hours and fungal plates were incubated at 30^0C for 72 hours. Inhibition zones in diameters were measured in mm using a calibrated calliper.

Metal toxicity test for isolated microorganisms: The metal toxicity test for different bacterial and fungal isolates were done against three different heavy metals such as Cadmium (Cd), Mercury (Hg) and Lead (Pb) at different concentration such as 1mM, 5mM, 10mM, 20mM. The metal toxicity was assayed for 24 hours, 48 hours and 72 hours for bacterial isolates and 48 hours, 96 hours and 144 hours for fungal isolates respectively for all the concentrations of the different heavy metals. The absorbance was calculated for each parameters at 620nm for bacterial isolates and 405nm for the fungal isolates by a double beam UV-VIS spectrophotometer with double distill water as blank (absorbance = 0) .

Results and Discussion

The bacteria were isolated and identified according to the colony morphology, different staining procedures and biochemical tests. From the four sampling points, 14 bacterial colonies were selected for the biochemical tests and their identification were done by Bergey's manual of determinative bacteriology as in table-1.

Different fungal species were isolated basing upon their observation in Lactophenol cotton blue staining and their colony morphology on the plates such as hyphae structure, colour, etc. Five fungal colonies were isolated as *Aspergillus niger, Aspergillus flavus, Penicillum species., Cladosporium* and *Fusarium.*

The different microorganism were analysed for the antibiotics sensitivity test where zone of inhibition (in mm) was calculated against antibiotics such as Ampicillin, Tetracycline, Chloramphenicol, Kanamycin, Erythromycin, Streptomycin and Nalidixic acid. table-2, figure-1 and figure-2.

The different microbial isolates were subjected to heavy metal toxicity test against three heavy metals such as Cadmium (Cd) as in figure-3 and figure-4, Mercury (Hg) as in figure-5 and figure-6 and Lead (Pb) as in figure-7 and figure-8 at different concentration such as 1mM in table-3, 5mM in table-4, 10mM in table-5 and 20 mM in table – 6 for three consecutive days for bacterial species and six consecutive days for fungal species.

Discussion: Different bacterial and fungal colonies isolated from different industrial sites were analyzed for their ability against antibiotics and tolerance against heavy metals such as Cadmium (Cd), Mercury (Hg) and Lead (Pb). While testing the antibiotics sensitivity test of isolates, *B.megaterium* showed highest zone of inhibition of 44mm with Erythromycin while *Fusarium sp.* showed highest zone of inhibition against Kanamycin. Highest resistance was seen in case of *S.saprophyticus* against Nalidixic acid while *Penicillum sp.* was sensitive against Chloramphenicol. Erythromycin was the potential antibiotics as it has good sensitive reaction against *P.fluorescence* (41mm)*, P.syringae* (31mm)*, B.subtilis* (42mm) while Tetracycline was better antimicrobial activity against *A.niger* and *A.flavus*. For Metal toxicity test against Cadmium, bacterial colonies such as *B.licheniformis, M.varians, S.saprophyticus, P.fluorescence, C.xerosis, B.insolitus, B.megaterium* proved better isolates. Fungal colonies such as *Fusarium, A.niger and A.flavus* were well tolerant to Cadmium toxicity at higher concentration. *P.fluorescence, B.megaterium, B.licheniformis, P.syringae, B.subtilis, C.xerosis, B.macerans* were effective at higher concentration (20mM) of Mercury toxicity. Likewise *Aspergillus niger, Fusarium, Aspergillus Flavus* were well-tolerant to Mercury toxicity. Bacteria that were tolerant to Lead toxicity at higher level (20mM) were *B.megaterium, B.licheniformis, P.fluorescence, S.saprophyticus, P.syringae, B.macerans, B.subtilis. Fusarium, A.niger, Cladosporium* showed good tolerant result for the Lead toxicity.

Conclusion

The different isolates that have a good tolerance level of heavy metal toxicity against test heavy metals such as Cadmium, Lead and Mercury can be good potentials for the bioremediation and the genes and genetics of those bacteria can be well exploited in future at molecular level to prove as a bio-machine against environmental pollution.

Reference

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Table-1
Biochemical test for identification of bacterial isolates

Biochemical test	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	**C7**	**C8**	**C9**	**C10**	**C11**	**C12**	**C13**	**C14**
Gram staining	+ve rod	+ve rod	-ve rod	-ve rod	+ve rod	-ve rod	+ve rod	+ve rod	+ve rod	+ve rod	+ve coccus	-ve rod	+ve rod	+ve rod
Endospore staining	**-ve**	**-ve**	*NA	*NA	**+ve**	*NA	**+ve**	**+ve**	**-ve**	**+ve**	*NA	*NA	*NA	**-ve**
Acid Fast staining	**-ve**	**-ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Catalase test	**+ve**	**+ve**	*NA	*NA	*NA	*NA	**+ve**	**+ve**	*NA	*NA	**+ve**	*NA	**+ve**	*NA
Mannitol fermentation test	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	**-ve**	*NA	**-ve**	*NA
Glucose fermentation	*NA	*NA	**+ve**	**+ve**	**+ve**	**-ve**	*NA	**+ve**	*NA	*NA	**+ve**	**-ve**	*NA	*NA
VP test	*NA	*NA	**-ve**	**+ve**	**-ve**	*NA	**-ve**	**-ve**	**+ve**	**+ve**	*NA	*NA	*NA	**-ve**
Indole test	*NA	*NA	**-ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Amylase production test	**- ve**	**+ve**	*NA	*NA	**+ve**	*NA	**-ve**	**-ve**	**+ve**	**+ve**	*NA	*NA	*NA	**+ve**
Motility test	*NA	*NA	**-ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Citrate test	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	**+ve**	**+ve**	*NA	*NA	*NA	**+ve**
Nitrate reduction test	*NA	*NA	*NA	*NA	*NA	**+ve**	**+ve**	*NA	*NA	*NA	*NA	**-ve**	*NA	*NA
Lecithinase test	*NA	*NA	*NA	*NA	*NA	**+ve**	*NA	*NA	*NA	*NA	*NA	**-ve**	*NA	*NA
Oxidase Test	*NA	*NA	**+ve**	**+ve**	*NA	**+ve**	*NA	*NA	*NA	*NA	*NA	**-ve**	*NA	*NA
6.5 % NaCl Test	*NA	*NA	**-ve**	**+ve**	**+ve**	*NA	*NA	*NA	**+ve**	**+ve**	*NA	*NA	*NA	*NA
Urease Test	*NA	*NA	**+ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Luminiscent agar Test	*NA	*NA	*NA	**-ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Pour plate	*NA	*NA	*NA	*NA	**-ve**	*NA	**-ve**	*NA	*NA	*NA	*NA	*NA	*NA	*NA
Pseudo P agar	*NA	*NA	*NA	*NA	*NA	**+ve**	*NA	*NA	*NA	*NA	*NA	**+ve**	*NA	*NA
Growth at 55^0C	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	**+ve**	**-ve**	*NA	*NA	*NA	*NA
Novobiocin sensitivity	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	**-ve**	*NA
Pigmented colony 37^0C	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	*NA	**+ve**	*NA	**-ve**	*NA
Identified micro-organism	*Corynebacterium xerosis*	*Corynebacterium kutsceri*	*Aeromonas caviae*	*Vibrio alginolyticus*	*Bacillus macerans*	*Pseudomonas fluorescence*	*Bacillus pasteurii*	*Bacillus insolitus*	*Bacillus licheniformis*	*Bacillus subtilis*	*Micrococcus varians*	*Pseudomonas syringae*	*S. saprophyticus*	*Bacillus megaterium*

+ve – organisms show positive result to test; **-ve** - organisms do not show results. *NA- biochemical tests/methodology not acquired or required.

Table-2
Antibiotics sensitivity test of isolated microorganisms

	Zone of Inhibition (in mm)						
Micro-organism	**Ampicillin**	**Tetracyclin**	**Chloramphenicol**	**Kanamycin**	**Erythromycin**	**Streptomycin**	**Nalidixic acid**
Bacterial Isolates							
C.xerosis	35 mm	27 mm	34 mm	36 mm	18 mm	42 mm	38 mm
C.kutsceri	40 mm	31 mm	27 mm	17 mm	29 mm	35 mm	43 mm
A.caviae	08 mm	14 mm	42 mm	23 mm	30 mm	29 mm	32 mm
V.alginolyticus	22 mm	35 mm	24 mm	40 mm	16 mm	24 mm	23 mm
B. macerans	27 mm	28 mm	34 mm	41 mm	35 mm	22 mm	18 mm
P.fluorescence	12 mm	33 mm	13 mm	22 mm	41 mm	17 mm	04 mm
B.pasteurii	33 mm	23 mm	38 mm	33 mm	22 mm	43 mm	34 mm
B.insolitus	37 mm	29 mm	40 mm	25 mm	26 mm	15 mm	27 mm
B.licheniformis	29 mm	19 mm	14 mm	32 mm	33 mm	37 mm	29 mm
B.subtilis	25 mm	28 mm	34 mm	37 mm	42 mm	27 mm	37 mm
M.varians	19 mm	32 mm	39 mm	21 mm	25 mm	20 mm	19 mm
P.syringae	13 mm	19 mm	30 mm	20 mm	31 mm	22 mm	06 mm
S.saprophyticus	22 mm	39 mm	31 mm	38 mm	21 mm	43 mm	03 mm
B.megaterium	31 mm	30 mm	39 mm	26 mm	44 mm	36 mm	21 mm
Fungal Isolates							
A.niger	33 mm	42 mm	08 mm	24 mm	13 mm	12 mm	19 mm
A.flavus	26 mm	35 mm	07 mm	13 mm	20 mm	16 mm	14 mm
Penicillum	36 mm	39 mm	05 mm	22 mm	23 mm	22 mm	17 mm
Cladosporium	22 mm	21 mm	12 mm	15 mm	21 mm	19 mm	10 mm
Fusarium	29 mm	27 mm	15 mm	32 mm	18 mm	27 mm	26 mm

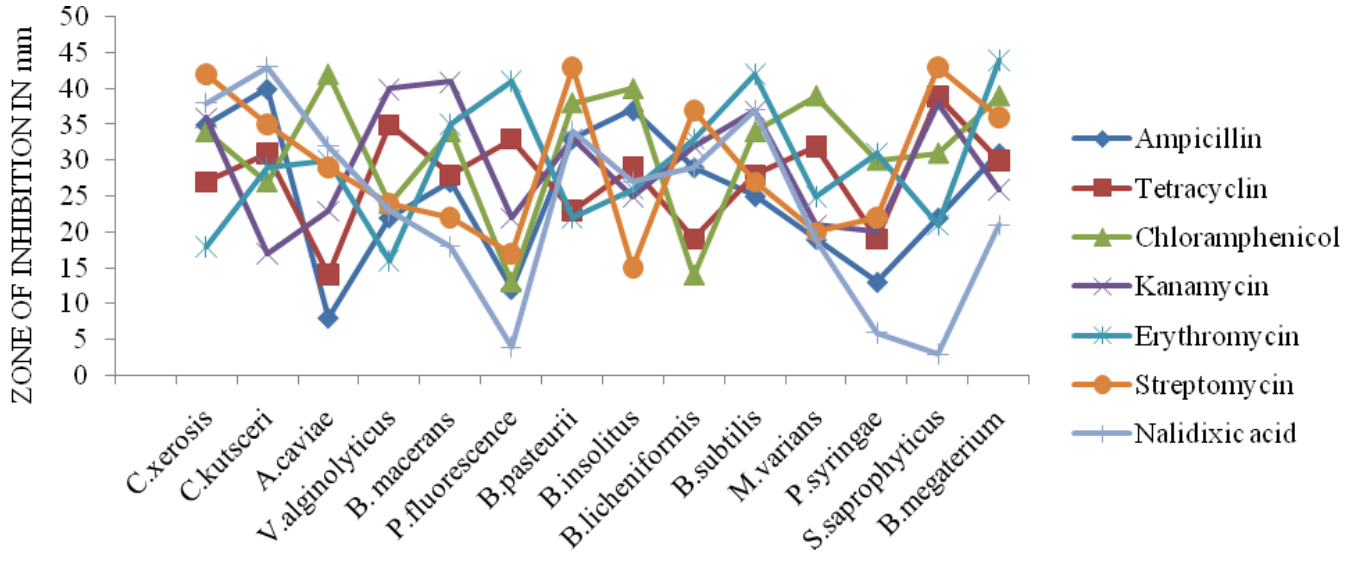

Figure-1
Antibiotics sensitivity test for different isolated bacterial species

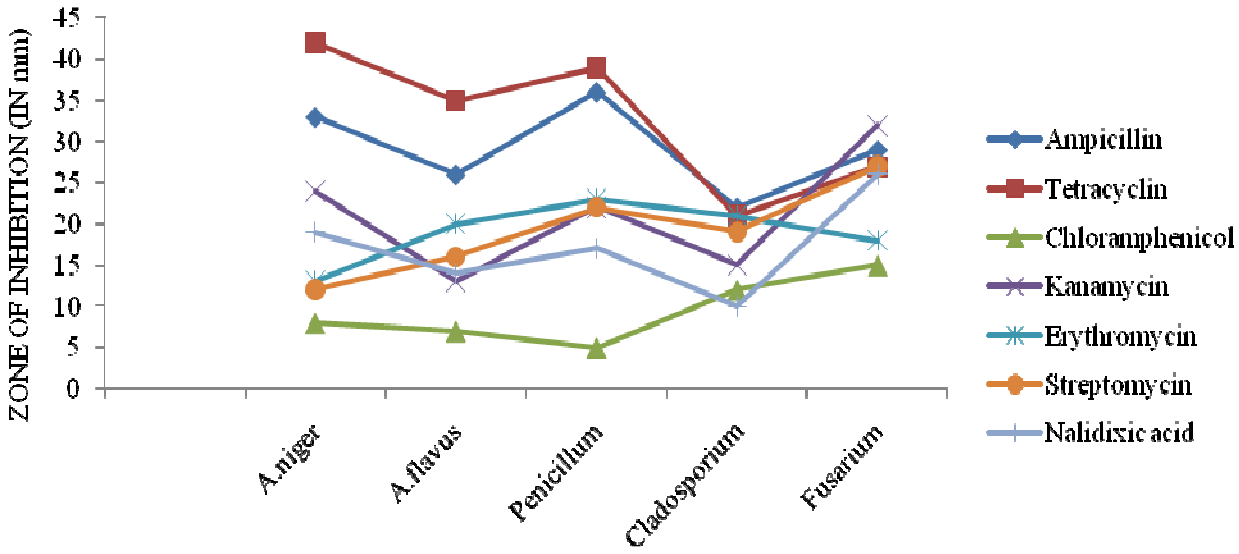

Figure-2
Antibiotics sensitivity test for different isolated fungal species

Table-3
Metal toxicity test against three heavy metals at 1mM concentration

	Absorbance at 620nm								
Heavy metals	Cadmium (Cd)			Mercury (Hg)			Lead (Pb)		
Bacterial Isolates									
Hours	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs
C.xerosis	0.31	0.37	0.42	0.23	0.31	0.39	0.36	0.44	0.52
C.kutsceri	0.24	0.27	0.30	0.22	0.28	0.33	0.33	0.40	0.48
A.caviae	0.25	0.29	0.33	0.21	0.25	0.29	0.31	0.34	0.39
V.alginolyticus	0.22	0.25	0.27	0.22	0.27	0.31	0.33	0.37	0.42
B. macerans	0.27	0.31	0.35	0.24	0.32	0.41	0.38	0.46	0.55
P.fluorescence	0.32	0.39	0.45	0.28	0.37	0.48	0.39	0.51	0.63
B.pasteurii	0.26	0.30	0.34	0.21	0.27	0.33	0.34	0.42	0.49
B.insolitus	0.30	0.36	0.42	0.23	0.29	0.34	0.35	0.44	0.53
B.licheniformis	0.34	0.42	0.50	0.25	0.34	0.42	0.41	0.53	0.65
B.subtilis	0.27	0.32	0.37	0.23	0.32	0.40	0.37	0.47	0.56
M.varians	0.33	0.43	0.49	0.23	0.30	0.39	0.30	0.32	0.34
P.syringae	0.28	0.34	0.39	0.26	0.35	0.32	0.38	0.49	0.58
S.saprophyticus	0.33	0.40	0.48	0.24	0.30	0.37	0.39	0.50	0.59
B.megaterium	0.29	0.35	0.41	0.27	0.35	0.45	0.42	0.55	0.64
Fungal Isolates (Absorbance at 405nm)									
Hours	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs
A.niger	0.25	0.29	0.34	0.27	0.32	0.37	0.28	0.32	0.38
A.flavus	0.24	0.28	0.31	0.22	0.26	0.31	0.21	0.25	0.28
Penicillum	0.21	0.23	0.26	0.21	0.23	0.26	0.23	0.25	0.32
Cladosporium	0.20	0.21	0.22	0.20	0.22	0.24	0.27	0.31	0.36
Fusarium	0.26	0.31	0.35	0.25	0.29	0.35	0.30	0.36	0.41

Table-4
Metal toxicity test against three heavy metals at 5mM concentration

	Absorbance at 620nm								
Heavy metals	Cadmium (Cd)			Mercury (Hg)			Lead (Pb)		
Bacterial Isolates									
Hours	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs
C.xerosis	0.28	0.33	0.37	0.21	0.28	0.34	0.32	0.41	0.48
C.kutsceri	0.22	0.23	0.22	0.20	0.25	0.31	0.32	0.36	0.40
A.caviae	0.22	0.21	0.22	0.17	0.18	0.18	0.29	0.30	0.31
V.alginolyticus	0.21	0.21	0.20	0.18	0.19	0.21	0.31	0.32	0.34
B. macerans	0.25	0.31	0.35	0.22	0.29	0.36	0.36	0.44	0.54
P.fluorescence	0.29	0.34	0.39	0.26	0.34	0.43	0.37	0.49	0.60
B.pasteurii	0.23	0.26	0.29	0.19	0.24	0.27	0.32	0.37	0.41
B.insolitus	0.28	0.31	0.35	0.21	0.26	0.31	0.33	0.40	0.48
B.licheniformis	0.31	0.38	0.46	0.23	0.31	0.39	0.39	0.52	0.65
B.subtilis	0.25	0.29	0.31	0.21	0.28	0.35	0.35	0.44	0.53
M.varians	0.30	0.36	0.42	0.21	0.27	0.30	0.28	0.28	0.29
P.syringae	0.27	0.30	0.33	0.24	0.31	0.38	0.36	0.47	0.58
S.saprophyticus	0.29	0.35	0.41	0.22	0.28	0.33	0.37	0.48	0.60
B.megaterium	0.27	0.31	0.35	0.25	0.33	0.41	0.40	0.53	0.65
Fungal Isolates (Absorbance at 405nm)									
Hours	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs
A.niger	0.24	0.29	0.32	0.25	0.30	0.36	0.25	0.29	0.33
A.flavus	0.23	0.25	0.28	0.20	0.24	0.29	0.19	0.24	0.26
Penicillum	0.18	0.19	0.20	0.18	0.20	0.26	0.21	0.23	0.25
Cladosporium	0.19	0.18	0.20	0.17	0.18	0.20	0.24	0.27	0.31
Fusarium	0.25	0.32	0.34	0.23	0.27	0.32	0.27	0.32	0.38

Table-5
Metal toxicity test against three heavy metals at 10mM concentration

	Absorbance at 620nm								
Heavy metals	Cadmium (Cd)			Mercury (Hg)			Lead (Pb)		
Bacterial isolates									
Hours	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs
C.xerosis	0.25	0.29	0.32	0.18	0.21	0.23	0.29	0.34	0.38
C.kutsceri	0.20	0.21	0.20	0.17	0.18	0.17	0.29	0.31	0.33
A.caviae	0.20	0.21	0.21	0.16	0.15	0.15	0.26	0.25	0.25
V.alginolyticus	0.21	0.20	0.20	0.16	0.16	0.15	0.28	0.29	0.30
B. macerans	0.22	0.22	0.23	0.19	0.22	0.24	0.33	0.45	0.53
P.fluorescence	0.27	0.31	0.32	0.23	0.28	0.33	0.34	0.43	0.54
B.pasteurii	0.21	0.22	0.22	0.16	0.17	0.17	0.29	0.32	0.34
B.insolitus	0.24	0.27	0.30	0.18	0.20	0.22	0.30	0.35	0.39
B.licheniformis	0.28	0.33	0.37	0.20	0.24	0.26	0.36	0.47	0.57
B.subtilis	0.23	0.24	0.25	0.19	0.23	0.26	0.32	0.39	0.45
M.varians	0.27	0.32	0.35	0.17	0.19	0.20	0.25	0.26	0.26
P.syringae	0.24	0.25	0.27	0.21	0.25	0.27	0.33	0.40	0.48
S.saprophyticus	0.26	0.29	0.34	0.19	0.21	0.23	0.34	0.43	0.52
B.megaterium	0.24	0.26	0.29	0.22	0.27	0.32	0.37	0.48	0.59
Fungal Isolates (Absorbance at 405nm)									
Hours	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs
A.niger	0.22	0.28	0.30	0.24	0.28	0.31	0.23	0.26	0.30
A.flavus	0.20	0.24	0.27	0.18	0.22	0.25	0.17	0.17	0.19
Penicillum	0.16	0.17	0.18	0.16	0.18	0.19	0.18	0.20	0.23
Cladosporium	0.17	0.19	0.20	0.15	0.16	0.16	0.20	0.24	0.27
Fusarium	0.23	0.28	0.31	0.21	0.26	0.29	0.24	0.28	0.33

Table-6
Metal toxicity test against three heavy metals at 20mM concentration

	Absorbance at 620nm								
Heavy metals	Cadmium (Cd)			Mercury (Hg)			Lead (Pb)		
Bacterial Isolates									
Hours	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs	24 hrs	48hrs	72hrs
C.xerosis	0.23	0.24	0.26	0.18	0.20	0.22	0.27	0.26	0.27
C.kutsceri	0.20	0.21	0.20	0.15	0.16	0.16	0.27	0.26	0.26
A.caviae	0.20	0.21	0.21	0.16	0.15	0.16	0.26	0.26	0.25
V.alginolyticus	0.20	0.20	0.21	0.16	0.15	0.17	0.26	0.25	0.25
B. macerans	0.21	0.21	0.22	0.17	0.19	0.20	0.33	0.37	0.41
P.fluorescence	0.23	0.25	0.27	0.22	0.25	0.27	0.34	0.39	0.44
B.pasteurii	0.20	0.21	0.21	0.16	0.15	0.15	0.28	0.27	0.26
B.insolitus	0.23	0.25	0.26	0.16	0.16	0.15	0.28	0.28	0.27
B.licheniformis	0.27	0.30	0.34	0.20	0.23	0.25	0.36	0.41	0.46
B.subtilis	0.22	0.22	0.23	0.18	0.21	0.23	0.32	0.36	0.40
M.varians	0.25	0.28	0.30	0.15	0.16	0.15	0.25	0.25	0.26
P.syringae	0.23	0.24	0.25	0.19	0.21	0.23	0.33	0.38	0.43
S.saprophyticus	0.24	0.27	0.29	0.15	0.16	0.16	0.34	0.39	0.42
B.megaterium	0.22	0.24	0.24	0.21	0.24	0.26	0.37	0.48	0.59
Fungal Isolates (Absorbance at 405nm)									
Hours	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs	48 hrs	96hrs	144hrs
A.niger	0.19	0.22	0.24	0.21	0.24	0.28	0.18	0.20	0.22
A.flavus	0.17	0.19	0.21	0.18	0.22	0.25	0.16	0.16	0.17
Penicillum	0.16	0.17	0.16	0.16	0.16	0.17	0.16	0.17	0.19
Cladosporium	0.16	0.17	0.17	0.16	0.15	0.16	0.17	0.18	0.21
Fusarium	0.21	0.24	0.25	0.19	0.23	0.26	0.20	0.23	0.25

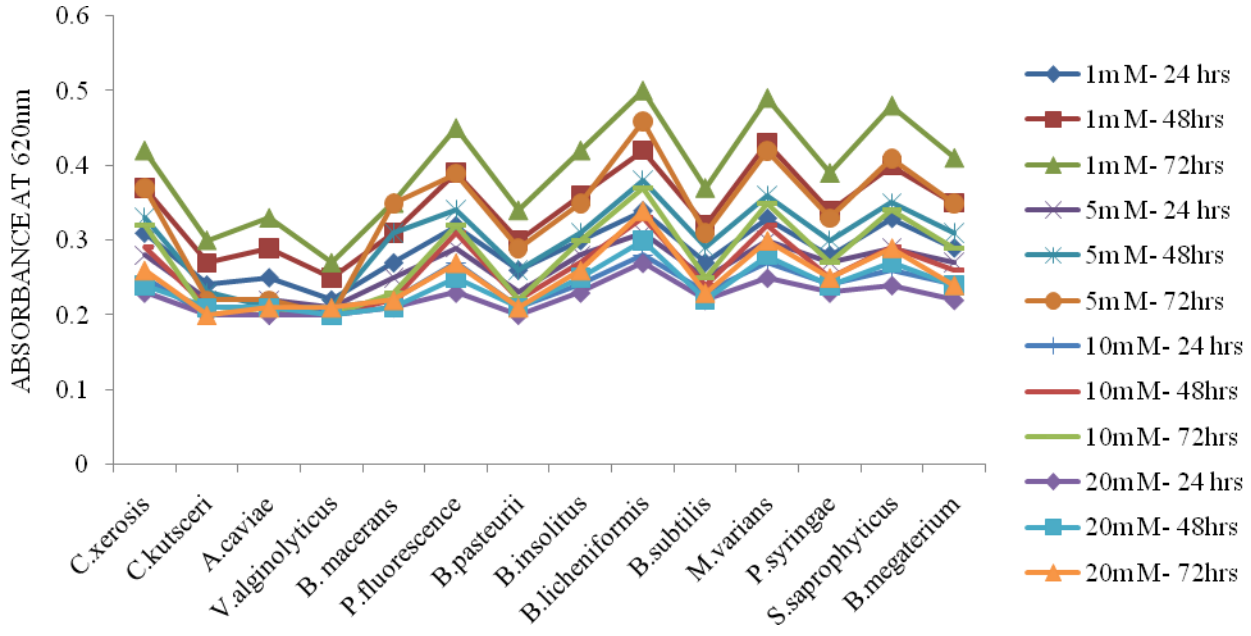

Figure-3
Comparative analysis for growth of different isolated bacterial strain against different concentration of Cadmium (Cd) at different time intervals

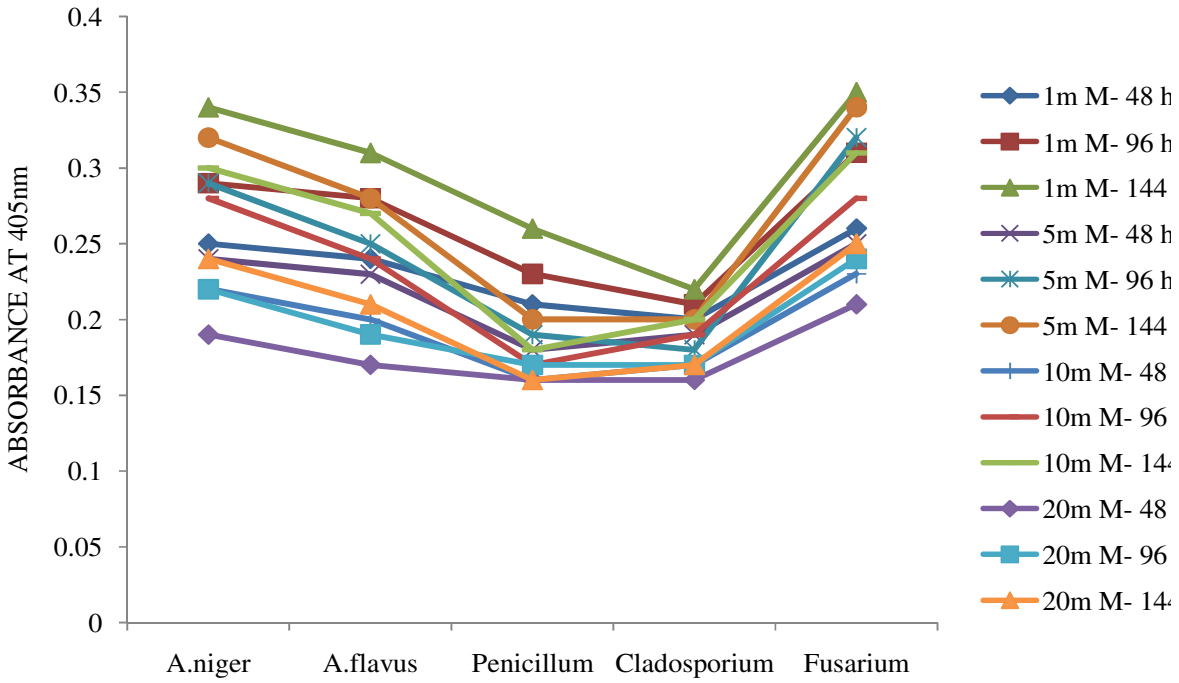

Figure-4
Comparative analysis for growth of different isolated fungal isolated strain against different concentration of Cadmium (Cd) at different time intervals

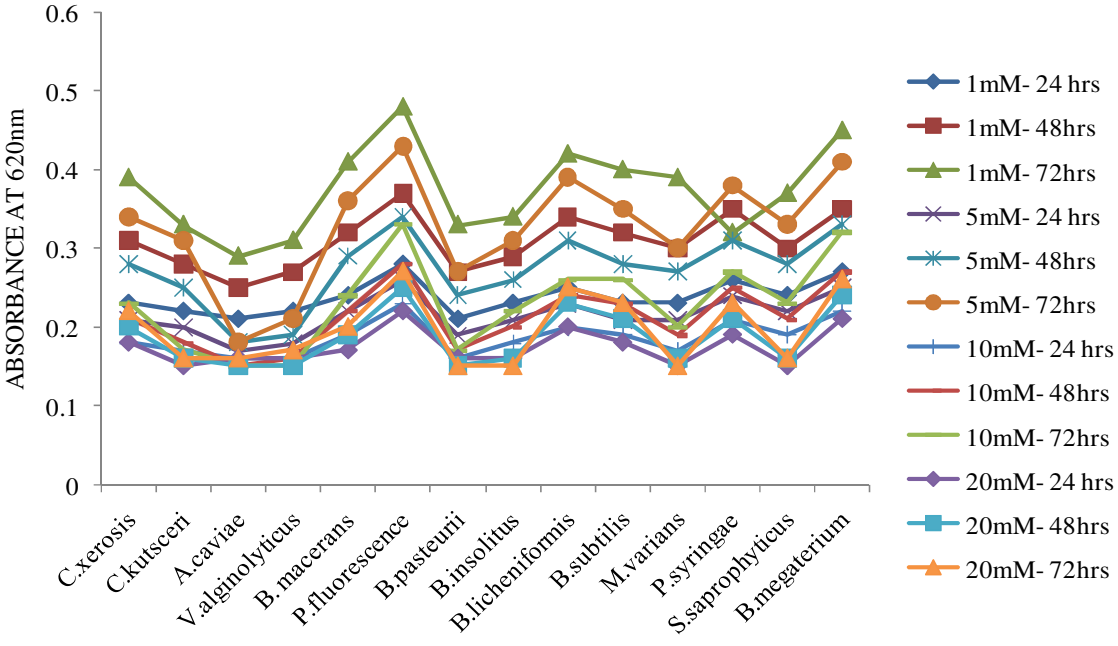

Figure-5
Comparative analysis for growth of different isolated bacterial strain against different concentration of Mercury (Hg) at different time intervals

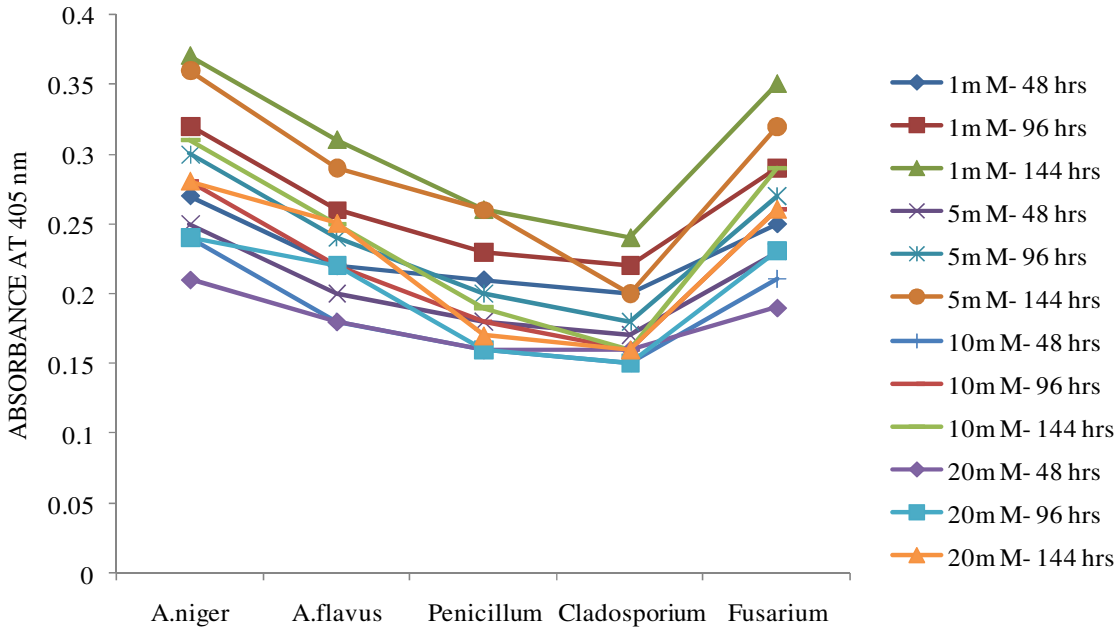

Figure-6
Comparative analysis for growth of different isolated fungal strain against different concentration of Mercury (Hg) at different time intervals

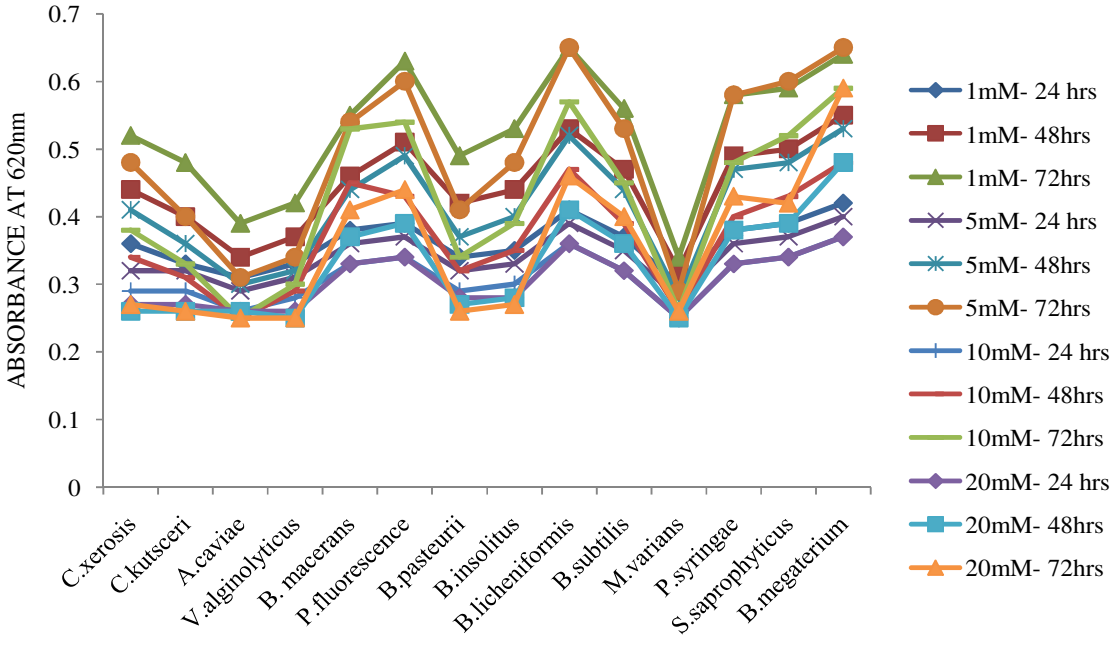

Figure-7
Comparative analysis for growth of different isolated bacterial strain against different concentration of Lead (Pb) at different time intervals

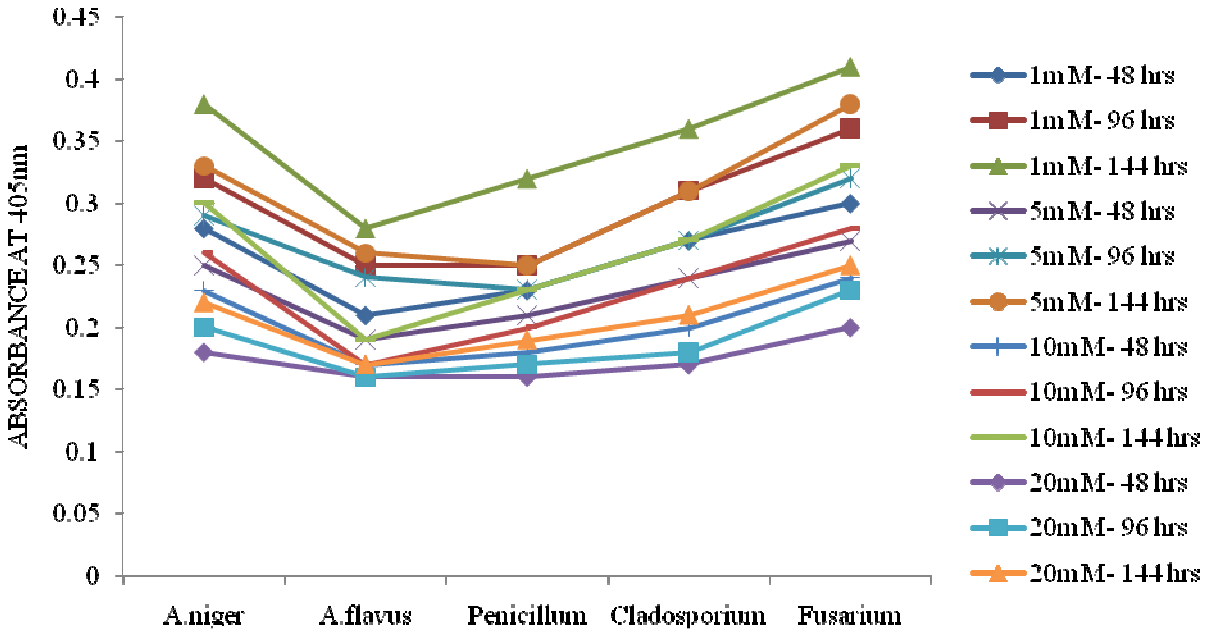

Figure-8
Comparative analysis for growth of different isolated fungal strain against different concentration of Lead (Pb) at different time intervals

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