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Accumulation of Copper in Industrial Soil: Contamination Status and Its Bioremediation.

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ABSTRACT

The concentration of Cu was measured in 128 samples of 4 different sites of industrial sites of district Rohtak (Haryana). The soil samples were collected for two years from area near to industries where solid waste, toxic substances were improperly disposed and also large amount of vehicular emission takes place. The aim of the present study was to study the pollution or contamination status of the soil as a result of anthropogenic activities and to identify the possible bioremediation of Cu in soil collected from industrial area, Hisar road,Rohtak (Haryana). It was found that site-BCSI in summer Cu contamination was more than other sites. It was also observed that at site-CI in summer season contamination was comparable to contamination in rainy season. Overall contamination at site-PI was less in all seasons. At site-PI, Cu accumulates less than 500 mg/Kg whereas at site-BCSI in summer contamination was higher than 5000 mg/Kg. Bacterial strains belonging to the bacillus family were isolated from industrial soil and result showed that these bacteria showed significant growth in 3% biomass concentration, maximum growth 20 mg/L of Cu and it gradually decreases upto 80 mg/L of Cu and remain same higher concentration. The result showed that these bacteria strain show significant growth in Cu environment in neutral conditions.

Keywords: Industrial soil; AAS; Cu contamination; bioremediation

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INTRODUCTION

Copper is naturally present in soils with range of 0 to 250 mg/g [1]. According to the literature the heavy metal concentration in urban and roadside soils is reported to be 5-10 times higher than the normal concentrations [2]. Copper is usually used in all the industries as electrical purposes and it would be change over from time to time and finally it would be discarded as one of the waste material. The copper content in the industrial area soils during wet season ranged from 6.8 mg/ Kg to 18.3 mg/Kg and varied up to 16.0 mg/Kg to 20.3 mg/Kg for dry season.

Copper in Environment

Copper is essential trace metal in human body, but if the body takes excessive Cu from environment, they will damage human health. Cu is tumor promoting factors, whose carcinogenesis effect has attracted global concerns.

Cu is used in numerous applications because of its physical properties. The toxicity for humans is not very high [3]. Cu normally accumulates in the bioaccumulation of the metal and recent anthropogenic sources. Contribution of Cu may be envisaged from dumping or accumulation of solid waste, application of fungicides, live stock manures, sludge and atmospheric deposition.

Copper in Soil

Copper is associated with organic matter, oxides of iron and manganese, silicate clays or adsorbed in soils and is one of the least mobile heavy metals at any pH. Organic matter is invariably the dominant factor controlling Cu retention. Most of the total dissolved Cu in surface soils occur in form of Cu^{2+} organic complex at higher pH condition. It builds up in the surface of contaminated soils showing virtually no downward migration. In alkaline soils Cu^{2+} solubility is very low and the solubility of total copper is enhanced by soluble complexes of Cu^{2+} (mostly hydroxyl, carbonate and organic matter complexes). Consequently, mobility may be significant under maximum pH condition [4]. A preliminary study and a detailed site survey indicated presence of contamination (mostly soil and broken battery cases). In addition to Cu surveys of urban areas, the spatial distribution of Cu concentration around industrial point sources of contamination has been investigated. The site selected for present study includes small scale industries located on Hisar road, Rohtak:

- **Site-1: Precision Fasteners Private Limited (PFPL)**
- **Site-2: Chemical Industry (CI)**
- **Site-3: Battery cases synthesising industry (BCSI)**
- **Site-4: Paint industry (PI)**

MATERIALS AND METHODS

Sampling

64 Soil samples from four different sites were collected quarterly in a year at surface level (5, 10, 15 and 20 cm depths) so as to cover industrial area near Hisar road, Rohtak. At each site, a 50-meter tape was laid parallel to the road (on both sides) of industry. Three quadrates (0.5 × 4 m) were placed at equal distances along the 50-meter tape in each zone. Samples from three quadrates of a zone were mixed together to make a composite sample representative of that zone of a particular site. The soil samples were taken from each quadrate at two points with a stainless steel auger from the top 5–20 cm of the soil. Large stones and plant materials were removed from soil samples. Samples were kept in a thoroughly pre-cleaned polyethylene bottles

Analysis of sample

The soil samples were dried at 110^0 C for 3 hours, ground to pass through a 2 mm mesh sieve and homogenized for analysis. A procedure recommended by environmental protection agency (EPA 3050B) was used as conventional extraction method. 1g of soil sample was heated to 95^0C with 10ml of 50% HNO_3 without boiling. After cooling the sample, it was refluxed with repeated addition of 65% HNO_3 until no brown fumes were given off by the sample. Then the solution was allowed to evaporate until the volume was reduced to 5 ml. After cooling 10 ml of 30% H_2O_2 was added slowly without allowing any losses. The mixture was refluxed with 37% HCl at 95^{0}C for 15 minutes. Distilled water was added and filtered. A clear solution was used for AAS measurement after dilution to 50 ml. The total extraction procedure was lasted for 100-200 minutes. The instrument was calibrated by using blank and five standards viz.2.5, 5.0, 7.5, 10.0, 12.5 ppm solution of Cu. After calibrating the instrument, the digested samples were analyzed to find the concentration of Cu.

Bioremediation of soil samples

Cu resistant bacterial strains were isolated from the industrial site soil samples using nutrient agar (NA) medium and was prepared using peptic digest of animal tissue (5g/l), beef extract (3g/l), NaCl (5g/L) and agar (15g/L). The isolated bacterial strain was amended with100 mg/L. Pour plate was performed in NA medium and was incubated at 37^{0}C.

Cu salt solution was prepared in different concentrations, say 10, 20, 40, 60, 80, 100 mg/L. After this the plate was spread with appropriate culture of the organisms. Then to the plate 100µL of Cu salt in well of 10 mm in diameter 4 mm in depths. Then NA plate was incubated at 37^{0}C for 24 hours. After incubation, the zone of inhibition was measured. Zone whose size was less than 1mm was scored as resistant strain.

Optimum conditions for Cu removal

Factors like temperature, pH, biomass, heavy metal concentration affects biosorption process. In series of test tubes the bacterial isolates were inoculated which contain 5ml of nutrient broth. pH varied between 5 to 9 (5, 6, 7, 8, 9) by adjusting the medium amended with 25 mg/L of Cu. The biomass concentration varied from 1 to 5% (1, 2, 3, 4 and 5) in a medium containing 25 mg/L of Cu. Concentration of Cu was varied from 20 to 100mg/L (20,40, 60, 80, and 100 mg/L).

RESULT AND DISCUSSION

Metal concentration in soil Samples

16 soil samples were collected at site -PFPL. In every season, 4 samples were collected of different depth as presented in table-1. In rainy season, Cu concentration was found maximum i.e. 3469.75 mg/Kg having standard deviation of 1247.64 whereas in summer season, Cu concentration was found minimum i.e. 201.77 mg/Kg having standard deviation of 113.20. In the same way 16 soil samples of 2013-14 of 4 different seasons were analyzed to find Cu concentration as presented in table-2. In rainy season, Cu concentration was found maximum i.e. 3509.36 mg/Kg having standard deviation of 1248.66 whereas in summer season, Cu concentration was found minimum i.e. 211.6 mg/Kg having standard deviation of 108.11.

Table-1: Concentration of copper in various seasons at site-PFPL in year (2012-2013)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	234.3	241.3	260.47	84.15
	10	126.2			
	15	318.05			
	20	286.65			
Spring	5	349.5	201.77	175.15	113.20
	10	231.2			
	15	119.1			
	20	107.3			
Summer	5	150.5	2089.5	1258.75	2593.67
	10	5690			
	15	239.5			
	20	2278			
Rainy	5	1888.5	3469.75	3579.75	1247.64
	10	3962			
	15	4831			
	20	3197.5			

Table-2: Concentration of copper in various seasons at site-PFPL in year (2013-2014)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	239.05	252.08	266.93	85.57
	10	138.4			
	15	336.05			
	20	294.8			
Spring	5	350.75	211.6	188.7	108.11
	10	243.2			
	15	134.2			
	20	118.25			
Summer	5	151.25	2096.88	1270.63	2595.66
	10	5695			
	15	243			
	20	2298.25			
Rainy	5	1932.6	3509.36	3614.43	1248.66
	10	4002.7			
	15	4876			
	20	3226.15			

In the similar way, 16 soil samples of year 2012-13were collected at site -CI. In every season, 4 samples were collected of different depth as presented in table-3. In rainy season, Cu concentration was found maximum i.e. 2058.85 mg/Kg having standard deviation of 1431.80 whereas in spring season, Cu concentration was found minimum i.e. 190.84 mg/Kg having standard deviation of 86.93. In the same way 16 soil samples for year 2013-14 of different seasons were analyzed to find Cu concentration as presented in table-4. In rainy season, Cu concentration was found maximum i.e. 2351.10 mg/Kg having standard deviation of 1446.54 whereas in summer season, Cu concentration was found minimum i.e. 199.41 mg/Kg having standard deviation of 88.98

Table-3: Concentration of copper in various seasons at site-CI in year (2012-13)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	433.25	235.98	216.72	154.84
	10	157.25			
	15	77.25			
	20	276.2			
Spring	5	255.25	190.84	187.17	86.93
	10	276.2			
	15	119.1			
	20	112.8			
Summer	5	1197.5	2035.06	2254.88	575.57
	10	2391.5			
	15	2433			
	20	2118.25			
Rainy	5	3354.5	2058.85	2339	1431.80
	10	2998.5			
	15	1679.5			
	20	202.9			

Table-4: Concentration of copper in various seasons at site-CI in year (2013-2014)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	439.25	245.11	228.15	152.84
	10	172.7			
	15	84.9			
	20	283.6			
Spring	5	268.2	199.41	197.63	88.98
	10	284.2			
	15	127.05			
	20	118.2			
Summer	5	1317.5	2096.67	2303.1	534.71
	10	2428			
	15	2463			
	20	2178.2			
Rainy	5	3426.8	2351.1	2351.1	1446.54
	10	3017.7			
	15	1684.5			
	20	228.45			

Again16 soil samples were collected from another site -BCSI. In every season, 4 samples were collected of different depth as presented in table-5. In summer season, Cu concentration was found maximum i.e. 5068.81 mg/Kg having standard deviation of 1625.00 whereas in spring season, Cu concentration was found minimum i.e. 121.65 mg/Kg having standard deviation of 22.20. In the same way 16 soil samples of different seasons of year 2013-14 were analyzed to find Cu concentration as presented in table-6. In summer season, Cu concentration was found maximum i.e. 5153.3 mg/Kg having standard deviation of 1668.18 whereas in spring season, Cu concentration was found minimum i.e. 124.74 mg/Kg having standard deviation of 23.2

Table-5: Concentration of copper in various seasons at site-BCSI in year (2012-2013)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	98.15	155.31	140.05	102.29
	10	54.5			
	15	181.95			
	20	286.65			
Spring	5	98.15	121.65	118.98	22.20
	10	150.5			
	15	125.3			
	20	112.65			
Summer	5	7270	5068.81	4631.63	1625.00
	10	3742			
	15	5315			
	20	3948.25			
Rainy	5	998.5	438.62	265.7	374.63
	10	224.6			
	15	234.3			
	20	297.1			

Table-6: Concentration of copper in various seasons at site-BCSI in year (2013-14)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	100.7	190.53	184.68	78.49
	10	180.25			
	15	189.1			
	20	292.05			
Spring	5	100.7	124.74	122.43	23.2
	10	153.4			
	15	112.3			
	20	132.55			
Summer	5	7410.5	5153.3	4710.6	1668.18
	10	3781.5			
	15	5410.5			
	20	4010.7			
Rainy	5	1007.65	433.76	271.58	385.53
	10	184.25			
	15	242.55			
	20	300.6			

Further16 soil samples were collected for year 2012-13 at site -PI. In every season, 4 samples were collected of different depth as presented in table-7. In rainy season, Cu concentration was found maximum i.e. 752.71 mg/Kg having standard deviation of 380.69 whereas in winter season, Cu concentration was found minimum i.e. 184.55 mg/Kg having standard deviation of 148.66. In the similar way, 16 soil samples of year 2013-14 of different seasons were analyzed to find Cu concentration as presented in table-8.In rainy season, Cu concentration was found maximum i.e. 911.45 mg/Kg having standard deviation of 619.91 whereas in winter season, Cu concentration was found minimum i.e. 189.71 mg/Kg having standard deviation of 151.23

Table-7: Concentration of copper in various seasons at site-PI in year (2012-2013)

Season	Depth (cm)	[Cu] in mg/kg	Mean	Median	Standard deviation
Winter	5	370.4	184.55	176.7	148.66
	10	14.4			
	15	213.35			
	20	140.05			
Spring	5	56.3	340.83	347.98	237.77
	10	611.5			
	15	433.25			
	20	262.3			
Summer	5	1250	733.47	792.87	491.49
	10	946.5			
	15	98.15			
	20	639.25			
Rainy	5	548.5	752.71	669.0	380.69
	10	789.5			
	15	1271			
	20	401.85			

Descriptive analysis

Data analyzed for different sites of four seasons (2012-13) were compared and it was found that site-BCSI in summer Cu contamination was more than other sites. It was also observed that at site-CI in summer season contamination was comparable to contamination in rainy season. Overall contamination at site-PI was less in all seasons. At site-PI, Cu accumulates less than 500 mg/Kg Whereas at site-BCSI in summer contamination was higher than 5000 mg/Kg shown in figure-1. In the similar way, data was analyzed for different sites of four seasons (2013-14) and compared. It was found that at site-BCSI in summer season Cu contamination was more than other two sites. It was also observed that at site-BCSI contamination was more in summer season but at site-PI contamination was less in all seasons. Overall contamination at site-PI was less in all seasons. Similarly it was observed that at site-PI, Cu accumulates little more than 500 mg/Kg whereas at site-BCSI in summer contamination was higher than 5000 mg/Kg shown in figure-2.

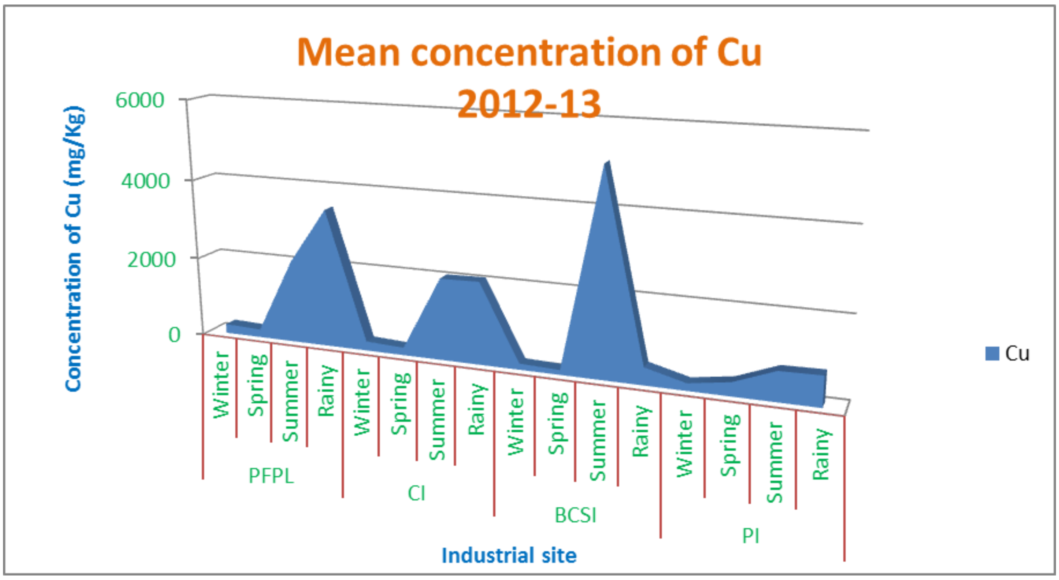

Figure-1: Copper Concentration at Various Industrial Sites in Various Seasons (2012-13)

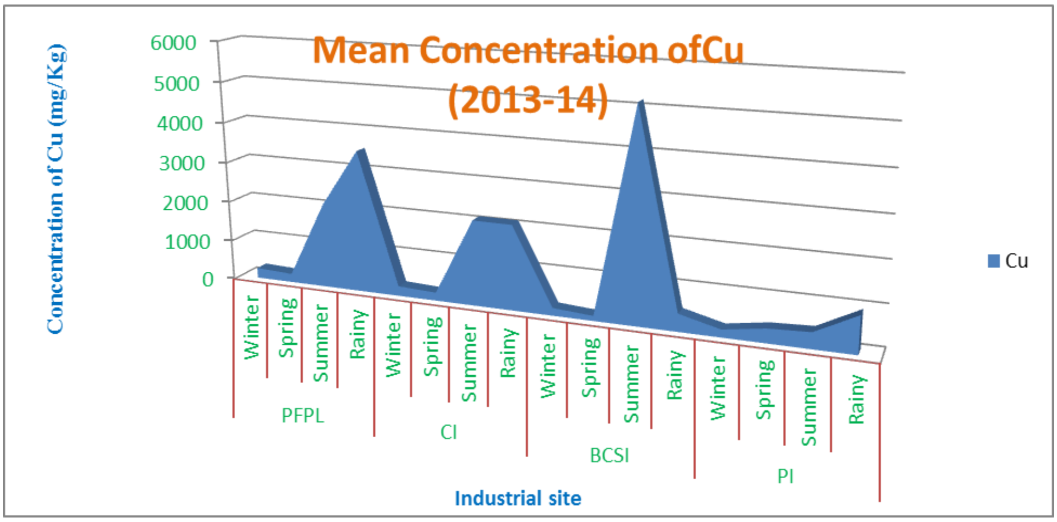

Figure-2: Lead Concentration at Various Industrial Sites in Various Seasons (2013-14).

Bioremediation of soil samples

In plate diffusion method, the results of zone formation show that Bacillus Sp. was resistant to Cu concentration. The identified strain was selected for further studies.

Optimum conditions for Cu removal

It was found that in the pH range studied (5 to 9) Cu resistant bacteria from bacillus family showed growth at initial pH 7 and decreased at increased pH as shown in figure-3. Simie et al.,[5] in 1998 found that biosorption by the cell depends on pH. Metal binding on the cell surface and their bioavailability depends on pH. At low pH cell surface binds to H^+ ions, thereby making the surface sites less available for binding of metal ions. However, on increasing pH binding of ligands increases with metal ions [6].

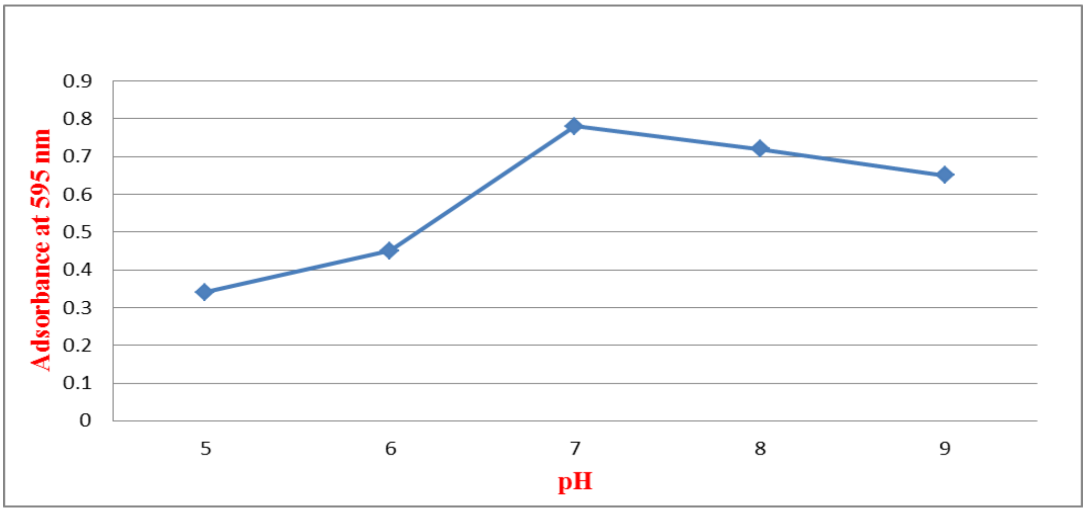

Figure-3: Cellular growth of Bacillus sp. in response to various pH. Temperature: 37°C, Incubation time: 24 hours.

Due to increase in pH, the negative charge on the surface of the cell increases which favored electrochemical attraction and adsorption of metal ions [7]. In the present study it was observed that Cu showed maximum growth at pH 7 which was similar to result of Wang and Chen (2006), [8] and Blackwell et al. (1995)[9]. The pH range 4-8 is widely accepted as optimal range for uptake of metal ions in all types of biomass.

It was observed that soil contaminated with Cu, Bacillus sp. showed maximum cellular growth at 3% of biomass as shown in figure-4. Thus, it was concluded from studies that as biomass concentration increases bacterial growth increases and further increase in biomass, decreases bacterial growth and decreases adsorption of metal ion on the surface of bacteria. In the previous study it was reported that high concentration of biosorbents cause cell agglomeration due to which intercellular distance decreases [10]. Therefore, that metal uptake will be higher when concentration of biosorbents is lower [11]. Similar results was observed in this study as represented in figure-5, Cellular growth of Bacillus sp. showed maximum growth 20 mg/L of Cu and it gradually decreases upto 80 mg/L of Cu and remain same higher concentration.

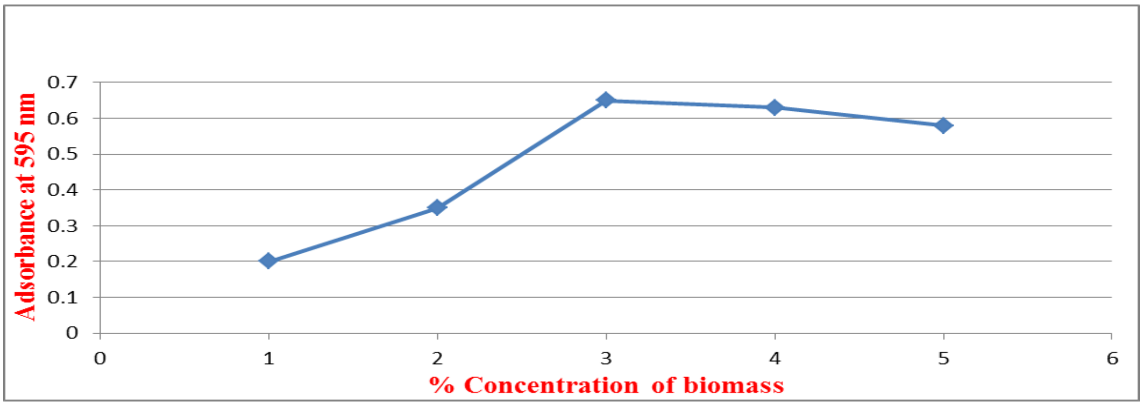

Figure-4 Cellular growth Bacillus sp.(bacterial isolate) in response with different biomass concentration. Temperature: 37°C, incubation time: 24 hours.

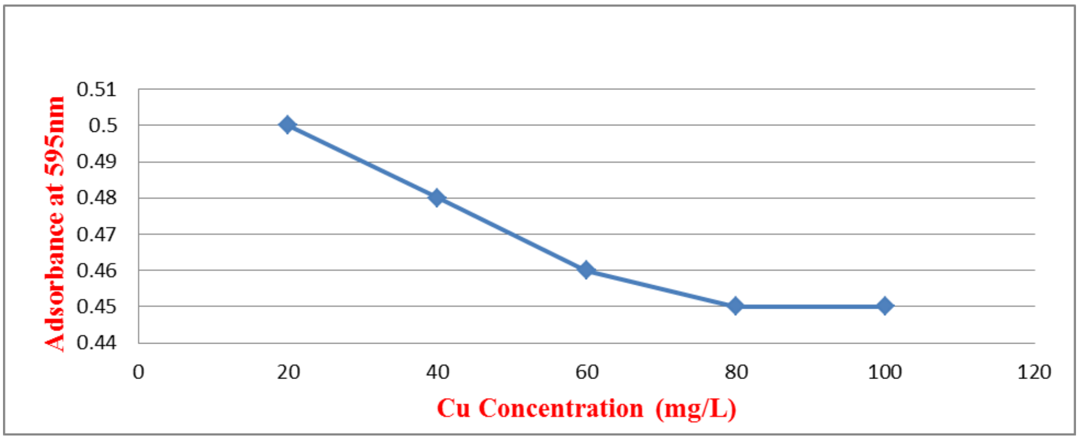

Figure-5: Cellular growth Bacillus sp.(bacterial isolate) in response with different Cu concentration (mg/L). Temperature: 37°C, incubation time: 24 hours.

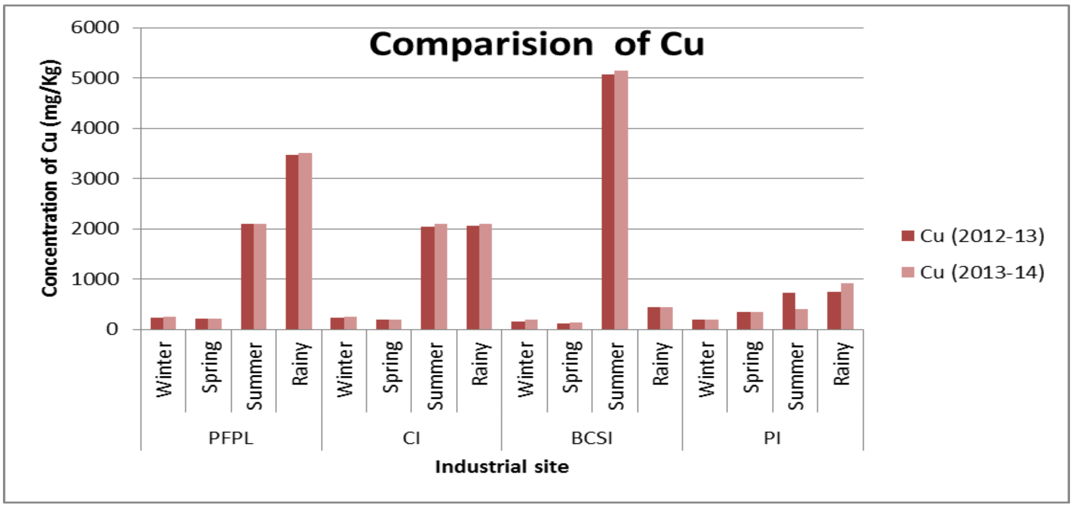

Figure-6: Comparison of copper contamination level for two years

CONCULSION

In research studies it was found that contamination level of Cu follows order: Site-BCSI > Site-PFPL > Site-CI > Site-PI and is shown in figure-6. Bacterial strains belonging to the bacillus family were isolated from industrial soil and result showed that these bacteria showed significant growth in 3% biomass concentration, maximum growth 20 mg/L of Cu and it gradually decreases upto 80 mg/L of Cu and remain same higher concentration. The result showed that these bacteria show significant growth in Cu environment in neutral conditions.

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