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BACTERIOLOGICAL ANALYSIS OF SOIL SAMPLES COLLECTED FROM FOUR CEMENT FACTORIES SIDES OF SATNA DISTRICT (M.P.)

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ABSTRACT

Totally 12 soil samples were collected from four cement factory areas *viz*.(Maihar cement factory, KJS cement factory, Reliance cement factory and Satna cement factory of Satna district Madhya Pradesh. Based on the area distribution, the soil samples were collected from three different sites A (inside the factory sample code A, D, G,& J), site B (2 km away in the area from the each factory, sample code B, E, H and K) and site C (5 km away in the area from the each factory, sample code C, F, I and L). The bacteriological analysis was carried out using standard methods. The highest bacterial count of $2629x10^4$ CFU/gm was observed in soil samples away 5 km from the cement factory in site C, while the least count of KJS cement factory soil sample away o km from KJS cement factory with micro-organisms load of $152x10^4$ CFU/gm. A total of 13 bacterial isolates were characterized and 8 fungus species were isolate and characterized. The results showed that conditions are not favorable for growth of microbs and plants.

KEYWORDS: Bacterial diversity, Cement factory, Soil, Cement factory, Dust pollution.

INTRODUCTION

Soil, air and water are major constituents of nature. Soil is composed of mainly five components such as air, water, mineral, organic material and living organisms. It is rich in organic matter and plays a role as mother of all living organisms including protozoa, algae, fungi, and bacteria. Soil is the thin layer of organic and inorganic materials [Sundararaj, 2004

& Obaroh *et al.*2016]. For agricultural activities and production of food materials fertile soils are necessary. But due various activities of humans soil can be polluted.

Soil pollution is defined as persistent of man-made toxic chemicals, salts, toxic compounds, disease causing agents and radioactive materials, which have adverse effects on environment, animal health as well as plant growth. Several various ways such as Indiscriminate use of fertilizer, pesticides, insecticides herbicides, discharge of industrial waste into the soil, Percolation of contaminated water into the soil, dumping of fuel and oil, dumping of large quantities of solid waste, rupture of underground storage tanks, soil erosion and deforestation, most common chemicals petroleum hydrocarbons, solvents, pesticides, lead and other heavy metals that soil can become polluted [Balinova, 19984 & Donkova, 2006].

For present study undertaken bacteriological analysis of soil samples in and around four cement factory of Satna district Madhya Pradesh. Maihar is a Tehsil of Satna district of Madhya Pradesh, India. It is well known for the temple of the revered mother goddess Sharda Devi and situated in Trikut hill. The town is well connected by road and rail. It is located on latitude 24.27^{o}N and longitude 80.75^{o}E. It has an average elevation of 376 meter (1204ft). There are three cement factories, *viz.* Maihar cement factory, KJS cement factory and Reliance cement factory. Maihar cement factory is situated at Sarlanagar about 8 Km. away from Maihar town on Maihar-Dhanwahi Road. The factory complex and the township are situated at Sarlanagar about 8 km away from Maihar town on the Dhanwahi road. Where KJS cement factory and Reliance cement factory are also located in Maihar Tehsil. Satna cement factory is located away from Maihar town 20 km in north –east region. Soil microorganisms can destroy pollutants, but pollutants can also destroy the some varieties of microorganisms. Thus the direct effects of cement dust pollution direct effect of the ecosystem soil chemical composition [Laj & Sellegri, 2003 and Mlitan *et al.* 2013]. Therefore, this study was carried out to assess the impact of cement dust pollution on the abundance and diversity of soil bacteria around a cement plant.

MATERIALS AND METHODS

Sampling of soil

For present study soil samples were collected in the area of four cement industries (Maihar cement factory, KJS cement factory, Reliance cement factory and Satna cement factory). Based on the area distribution, the soil samples collected from three different sites A (inside the factory sample A, D, G,& J), B (2 km away in the area from the each factory, sample B,

E, H and K) and site C (5 km away in the area from the each factory, sample C, F, I and L). Total 12 samples were collected from 12 different sites in clean sterile polythene bags.

Soil preparation and bacterial analysis

All 12 soil samples were sieved separately to remove large pieces of particles and debris. Serial dilutions were carried out by measuring 1g soil from each sample with a sterile spatula into a four 250 ml beaker under normal atmospheric condition. Then 12 ml of sterile water was introduced into each beaker and the soil suspension was stirred gently for 3 minutes to obtain a homogenized solution. Nine milliliter (9 ml) of sterile distilled water was later measured into 12 labeled test tubes. Afterward, 1ml was measured from the stock solution and dispensed into the first test tube labeled (12-1). From the first test tube, 1ml was introduced into the second test tube labeled (12-2), and continuously up to the last test tube label (12-12).The samples were cultured by using spread soil dilution plate method. Each dilution of the series (Nutrient agar, pH 7.2) was prepared and 20ml was placed onto petri-dishes containing nutrient agar and incubation at 37^{o}C for 24-hrs. After the incubation, the average colony forming units (CFU) per gram of soil from three different plates were calculated [Madigon, & Martinko, 2006].

Isolation and Identification of Microbes

To obtain pure cultures, colonies of bacteria and fungi which different in colour and shaped were picked up and purified by streaking fresh on nutrient agar and incubated at 37^{o}C for 24 hrs. The bacterial isolates were identified by Gram staining and other characteristics on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology [Madigon, & Martinko, 2006 and Krieg, & Holt, 1984].

Biochemical Characterization of Bacteria

Isolated bacteria were characterized using by biochemical tests *viz.* Voges proskauer (MR-VP), Indole test, coagulase test, and triple sugar iron agar test (TSI), as described by various authors [Ogbulie, 1998 and Cheesbrough, 1999].

Citrate Utilization Test

The test organism was inoculated on a Simmons citrate agar (SCA) slant with a sterile wire loop. The tube was inoculated at 35^{0}C for twenty four to twenty forty eight (24-48) hours before examination. The presence of a blue colour indicated a positive test for citrate

utilization, while the absence of growth and a green colour indicated negative test [Ogbulie, 1998 and Cheesbrough, 1999].

MR-VP Test

In this test, 10 ml of MR-VP medium in a test tube was inoculated with the test organism and incubated at 35^0C for twenty four to twenty eight (24-48) hours. Five drops of methyl red indicator solution was later added to 5ml of the culture. The appearance of red colour indicates the positive methyl red test, while yellow colour represents a negative test. To the remaining 5ml of the culture, 0.6ml of napthol solution and 0.2ml of 40% potassium hydroxide solution was added and kept for 2 to 4 hours. The appearance of red color indicated the positive VP test (Voges proskauer test), while yellow indicated negative test [Ogbulie, 1998].

Indole Test

One percent (1%) tryptophan broth in a test tube was inoculated with a bacteria colony. After incubation period of 37^oC for 48 hours, 1 ml of chloroform was added to the broth. The test tube was shaken gently, after which 2 ml of Kovac's reagent was added. Again, the test tube was shaken gently and allowed to stand for twenty (20) minutes. The formation of red colour at the top layer means positive while yellow coloration means negative test [Ogbulie, 1998 and Cheesbrough, 1999].

Triple-Sugar Iron Agar Test (TSI)

The medium contains three (3) sugars namely: glucose, lactose and sucrose. The PH indicator is phenol red, and detection system for hydrogen sulphide (H_2S) is included. This medium was prepared as agar slope and the test organism was inoculated by stabbing the medium with the aid of sterilized straight wire loop. The surface of the slope is inoculated by streaking and then incubated at 37^oC for 24 hours, after which observation was made. Gas production was determined by cracking of the medium. The formation of H2S was determined by the blackening of the whole buffer or a streak of ring of blackening at the slant butt junction. Glucose fermentation was determined by the yellowing of the butt. The fermentation of lactose or sucrose or both was determined by the yellowing of both the bottom and the slant. The motility was determined by observing the line inoculation; sharply defined line of inoculation indicated positive motility [Cheesbrough, 1999].

RESULTS AND DISCUSSION

Bacterial Load in Soil Sample

The highest number of colonies of micro-organisms 2629x10^4 CFU/gm (where total no of bacteria 2625x10^4 CFU/gm and total no. of fungi 4x10^4 CFU/gm) load was observed in the Maihar cement factory soil sample away 5km from Maihar cement plant while the least was observed in KJS cement factory soil sample away o km from KJS cement factory with micro-organisms load of 152x10^4 CFU/gm (where total no of bacteria 150x10^4 CFU/gm and total no. of fungi 2x10^4 CFU/gm**)** as shown in given table. 12 sampling stations soil sample tests results are given in Table -1

Table 1: Bacteria and fungi count in soil samples CFU/gm (x 10^4).

S. no.	Name of sampling stations	Distance from factory	Samples code	No of colonies of micro-organisms	No. of bacteria	No. of fungi
1	Maihar cement factory	0km	A	255	255	0
2	Maihar cement factory	2km	B	1536	1535	1
3	Maihar cement factory	5km	C	2629	2625	4
4	KJS cement factory	0km	D	152	150	2
5	KJS cement factory	2km	E	235	235	0
6	KJS cement factory	5km	F	306	295	11
7	Reliance cement factory	0km	G	422	412	10
8	Reliance cement factory	2km	H	459	450	9
9	Reliance cement factory	5km	I	2082	2050	32
10	Satna cement factory	0km	J	420	410	10
11	Satna cement factory	2km	K	421	411	11
12	Satna cement factory	5km	L	443	430	13

Biochemical Characterization and Gram reaction of Isolated Bacteria from 4 Cement industry sides 12 soil samples

Biochemical characterization and gram reaction of isolated bacteria from 4 cement industry sides, 12 soil samples were performed. Results are given in table -2.

Table 2: Showing biochemical characterization and gram reaction of Isolated bacteria and fungi from cement industry soil.

S. no.	Name of sampling stations	Distance from factory	Samples code	Biochemical tests					Shape	Gram stain	Colony characteristics	Identified organism	
				MR	VP	Ctr	Ind	TSI				Bacteria	Fungus
1	Maihar cement factory	0km	A	--	--	--	--	-	*Cocci*	-ve	Round, Smooth, Smooth	*Non identified*	*Penicillium Spp.*
				--	--	+	--	--	*Bacilli*	-ve	Filamentous filamentous, Smooth	*B. subtilis*	
2	Maihar cement factory	2km	B	--	--	+	--	--	*Cocci*	-ve	Irregular,Lobate,Countered	*P.aeroginosa*	*No fungal growth*
				+	--	+	--	--	*Bacilli*	-ve	Round, Smooth,Concentric	*Non identified*	
				+	--	+	+	-	*Bacilli*	+ve	Irregular,Smooth,Wrinkled	*Providenella spc.*	
3	Maihar cement factory	5km	C	+	+	+	--	--	*Bacilli*	-ve	Round,Smooth,Smooth	*Non identified*	*Penicillium Spp.*
				+	+	+	--	--	*Bacilli*	+ve	Round,Smooth,Smooth	*Non identified*	
				+	--	--	--	--	*Bacilli*	+ve	Round,Smooth,Concentric	*Salmonella typhi / S.paratyphi*	
4	KJS cement factory	0km	D	+	+	--	--	-	*Cocci*	-ve	Filamentous,Lobate,Smooth	*Non identified*	*No fungal growth*
				+	+	+	--	+	*Bacilli*	+ve	Round,Smooth,Concentric	*Non identified*	
				+	+	+	--	--	*Bacilli*	-ve	Round, Concentric, Wrinkled	*Non identified*	
5	KJS cement factory	2km	E	+	+	+	--	--	*Cocci*	+ve	Round,Smooth,Concentric	*Non identified*	*Aspergillus fumigatus, Aspergillus spp.*
				+	+	+	--	-	*Cocci*	-ve	Round,Smooth,Smooth	*Non identified*	
				--	--	--	--	-	*Bacilli*	-ve	Round,Smooth,Concentric	*Non identified*	
6	KJS cement factory	5km	F	+	+	+	--	+	*Cocci*	-ve	Round,Curled,Wrinkled	*Non identified*	*Fumigatus,penicillium Aspergillus niger*
				+	+	+	--	-	*Bacilli*	+ve	Irregular,smooth,Smooth	*Non identified*	
				--	--	--	--	--	*Bacilli*	+ve	Round,Smooth,Smooth	*Shigella spc.*	
				+	+	+	--	+	*Bacilli*	+ve	Filamentous,Lobate,Wrinkled	*S.typhi*	
				--	--	--	--	--	*Bacilli*	-ve	Irregular,Curled,Wrinkled	*Non identified*	
7	Reliance cement factory	0km	G	+	+	--	--	--	*Cocci*	-ve	Round,Smooth,Smooth	*Non identified*	*Mostly Aspergillus spp.*
				--	+	--	--	--	*Cocci*	+ve	Irregular,Curled,Wrinkled	*Proteus mirsbilis*	
				+	--	+	--	--	*Bacilli*	+ve	Round,Smooth,Smooth	*B.subtilis*	
				--	--	+	--	--	*Bacilli*	+ve	Round,Smooth,Smooth	*S.aureus*	

				--	--	--	--	--	*Cocci*	-ve	Round,Smooth,Smooth	*Shigella spc.*	
8	Reliance cement factory	2km	H	+	--	--	--	--	*Bacilli*	-ve	Round,Curled,Wrinkled	*Non identified*	*Fusarium, Green mold*
				+	+	+	--	+	*Cocci*	-ve	Round,Curled,Wrinkled	*Non identified*	
				--	+	--	--	--	*Cocci*	-ve	Irregular,Curled,Smooth	*Non identified*	
				--	--	--	+	--	*Bacilli*	+ve	Round,Wavy,Wrinkled	*Non identified*	
9	Reliance cement factory	5km	I	+	--	--	--	--	*Cocci*	+ve	Irregular,Lobate,Smooth	*E.coli*	*Rhizopus.Mucor, Cladosporium*
				--	--	+	+	-	*Bacilli*	-ve	Irregular,Lobate,Concentric	*Non identified*	
10	Satna cement factory	0km	J	+	+	+	+	--	*Bacilli*	+ve	Round,Smooth,Concentric	*Non identified*	*Aspergillus flavus,Green mold*
				--	+	--	+	-.	*Bacilli*	+ve	Filamentous,filamentous,Smooth	*Proteus mirsbilis*	
				--	+	--	--	--	*Mix*	-ve	Irregular,Wavy,Concentric	*Non identified*	
				--	+	--	--	-	*Cocci*	-ve	Irregular,Lobate,Wrinkled	*Non identified*	
				--	+	--	--	-	*Bacilli*	+ve	Round,Smooth,Concentric	*Non identified*	
11	Satna cement factory	2km	K	--	--	--	--	--	*Cocci*	+ve	Round, Smooth, Smooth	*Non identified*	*Trichoderma. Aspergillus*
				--	--	--	--	--	*Cocci*	-ve	Round, Smooth, Wrinkled	*M.luteus*	
				+	--	--	--	--	*Bacilli*	-ve	Round, Smooth,Smooth	*Salmonella paratyphi*	
				+	--	+	--	-	*Bacilli*	+ve	Punctiform, Curled, Smooth	*B.subtilis*	
				--	--	+	--	--	*Bacilli*	+ve	Round, Curled, Smooth	*Non identified*	
12	Satna cement factory	5km	L	+	--	+	--	--	*Bacilli*	-ve	Irregular,smooth,Wrinkled	*S.aureus*	*Mucor, mostly Aspergillus*
				+	--	--	--	--	*Bacilli*	-ve	Irregular,smooth,Smooth	*Salmonella typhi / S.aureus*	
				--	+	+	--	+.	*Bacilli*	-ve	Irregular, Curled, Concentric	*Proteus mirsbilis / B.subtilis*	

Abbreviations: +VE = Positive; -VE = Negative; MR = Methyl Red; VP = Vogue's Poaskauer; Ctr =Citrate; Ind = Indole; TSI= Triple-Sugar Iron Agar Test

The high bacteria and fungi densities at the soil samples compared with other soil samples in 0km 4 cement factory *viz.* Maihar cement factory, KJS cement factory, Reliance cement factory and Satna cement factory found in decreasing order. This could be because less soil pollution due to cement dust away 5km from Cement factories. The soils samples (soils samples away from 5km of Cement factory and free of cement dust) tests findings were observed to have higher bacterial load than those from cement dust populated area, this result is in agreement with [Adesemoye, *et al.,* 2006; and Stanley *et al.* 2014] who reported increase in microbial diversity and population as sample collections were moved away from the factory site. The bacterial isolates identified in this study were mostly represented by Gram-negative bacteria which were often found in 5km away from Cement factory soils, and this result is in agreement with the observation made by [Kulandaivel, *et al.* 2015].

CONCLUSION

Present study has established that low bacteria populations and diversity in the contaminated soils showed that cement dust is toxic to bacteria. These conditions are not in the favour of microbial diversity and soil fertility. Therefore, pollution control and prevention strategies must be follows to cement factories.

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