IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

BIODEGRADATION OF TRACE METALS BY BACTERIA

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1.ABSTRACT;

Heavy metal pollution has drawn increasing attention worldwide owing to a dramatic increase in anthropogenic heavy metals in ecosystems through air, water and soil (Woitke *et al*., 2003).The aim of this study was to determine the level of pollution indicators, concentrations of physiochemical, heavy metal and microbiological parameters from the certain oil contaminated regions (four sampling sites) of Tiruchirappalli city during one year (four different seasons-– post monsoon, summer, premonsoon and monsoon seasons).The spatial and temporal variations of all parameters (variables) and their interactions between these two things by using different statistical tools was analyzed physiochemical parameters, i.e., pH, electrical conductivity (EC), total dissolved solids (TDS) and salinity were measured by standard methods (APHA, 1998; Vignesh *et al*., 2014,.The isolated 60 bacterial strains from the study sites were challenged against chromium metal (Potassium chromate) solutions with four different concentrations (10 mm, 50 mm, 100 mm and 250 mm) for metal resistant studies by plate diffusion and tube dilution methods. The identified the selected potential MMR strains by molecular analysis (16s rDNA sequencing and phylogenetic tree construction studies) Based on the MMR results, potential strains (multi metal resistant strains) were used for bioremediation studies with dry biomass (Bio sorption) and living biomass studies. The copper removal potential of bacterial strain 1 was higher than bacterial strain 2 and also the same pattern follows in the chromium removal methods. Interestingly, the copper was highly removed by microbes than chromium. The bacterial strain 1 effectively removed the metals from a both field trail and natural (medium + metal solution) samples due to its higher metal tolerance, residual growth rate and efficient metal removal. The present results indicate that both *Pseudomonas* biomasses may be a suitable material for the removal of copper and chromium ions from the solution.

The dry biomass was act as an effective bio sorbent than the living bio sorbents. In both study, bacterial strain 1 was act as an effective bio sorbent than bacterial strain 2. However, several phases of metal–bacteria interactions remain unexplored and further improvement and application are necessary.

Keywords; Heavy metal,MMR,r-DNA Sequesing,Biosorption,TDS ,Phylogenetic analysis

1.INTRODUCTION;

Environmental surveys are necessary for understanding and documenting the occurrence and distribution of pollution indicator and human pathogenic bacteria. In order to quantify and understand their relationship with relevant environmental factors, several investigators have examined distribution of these groups of bacteria and certain viruses in coastal waters (Kumarasamy *et al*., 2009; Nagvenkar and Ramaiah *et al*., 2009; Vignesh *et al*., 2012). Heavy metal pollution has drawn increasing attention worldwide owing to a dramatic increase in anthropogenic heavy metals in ecosystems through air, water and soil (Woitke *et al*., 2003). As a result of increasing industrialization, water pollution due to heavy metals has posed serious problems in many aquatic systems since the bacteria can acquire resistance after exposure to these agents Aquatic bacteria develop its resistance behavior to adopt themselves to extreme environments including toxic heavy metals. It has been suggested that the metal resistance may not be a fortuitous phenomenon and bacterial resistance against heavy metals appears to be directly related to the presence of these elements as environmental pollutants

Metal removal by conventional methods like precipitation, flocculation, ion exchange and membrane filtration is expensive and not effective at low concentrations. Bioremediation is an emerging cost-effective, environmentally safe method for the cleanup of environments contaminated with heavy metals compared with conventional methods (De *et al*., 2006). Microorganisms that have been involved directly in pollutant degradation are principally bacteria, and to a lesser degree fungi, protozoa and benthic invertebrates. Microorganisms, especially bacteria from long-term polluted environments show resistance to several toxic metals Bacterial metal resistance may be mediated by genetic factors, the production of chelating materials (polysaccharides, proteins, etc.), binding by cell surface slime and/or oxidative detoxification (De *et al*., 2008). The resistance and metal removal efficiency of microbes vary greatly. The aim of this study was to determine the level of pollution indicators, physiochemical and heavy metal parameters from oil contaminated regions of Tiruchirappalli city. Certain strains were isolated from those regions and were challenged against different concentrations of 2 different metals (Copper and Chromium) for heavy metal resistant/ multi metal resistant studies. In addition, the study investigated the copper and chromium sorption capability (dry biomass and living cell) of two selected bacterial strains.

2. Methodology

2.1 Sampling and preprocessing

The water and soil sample were collected from the four different oil contaminated regions of Tiruchirappalli city during (four seasons – post monsoon, summer, premonsoon and monsoon seasons) one throughout year (2015). The 500 mL of oil contaminated water samples were collected with a 2500 mL sterile container in each locations. The oil contaminated soil samples (250 g) were collected by sterile spatula and stored in sterile plastic bags and stored in ice box at 4 °C (Kumarasamy *et al*., 2009; Vignesh *et al*., 2014). The samples were transported into laboratory and processed within 12 hrs (Vignesh *et al*., 2013; 2015). The sampling sites are Ponmalai Railway Shed (PRS), Senthaneerpuram Oil Shed (SOS), Chatram Bus Stand (CHB) and Central Bus Stand (CLB). The

sampling sites were choose based on the oil pollution. In which the sampling sites were divided in to two categories such as oil shed (PRS and SOS) and oil waste mixing with sewage (CHB and CLB).

2.2Physiochemical analysis

The physiochemical parameters, i.e., pH, electrical conductivity (EC), total dissolved solids (TDS) and salinity were measured using field kit (Thermo Orion 5-Star pH Multi-Meter) on the site and the concentrations of soluble cations, anions and nutrients (around 20 parameters) were determined according to the standard methods (APHA, 1998; Vignesh *et al*., 2014, 2015). All samples were collected with precautions required for all analysis, held on iceboxes and processed within 6 h of collection.

2.3 Trace metal analysis

The one liter of oil contaminated water was acidified immediately with concentrated nitric acid (HNO_3) after collection of the sample and were filtered by Whatman No.1 filter paper. After filtration, the sample was processed (APDC + MIBK) for metal analysis. The sediment samples were air-dried and smaller than (>) 63 µm in size were retained in pre-cleaned properly. Thereafter, the dried sediment samples were crushed by agate mortar and pestle. The crushed soil sample was treated with aqua-regia mixture (i.e. HCl:HNO_3= 3:1) in Teflon bomb and were incubated at 140 °C for 2-3 days after dried and sieved samples. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. The trace metals in the water and soil sample were determined by the atomic absorption spectrophotometry (GBC SensAA - AAS, Australia) in flame mode (Muthukumar *et al*., 2015).

2.4Bacteriological analysis

The bacterial populations in different samples were estimated by pure culture technique (spread plating method) on selective medium plates with 100 µL of suitable dilutions (Kumarasamy et al., 2009). In this study, the selective media were prepared with the addition of double distilled water and autoclaved properly. After addition of sample on selective media plates, the plates were incubated at 37°C ± 1°C for 24–48 h, except M-FC agar plates. The M-FC agar plates were incubated at 44.5°C ± 1°C for 24–48 h (Vignesh *et al*., 2013). After incubation, the final counts of colonies were noted and all trials were performed in triplicate. For confirmation of the pathogens, typical colonies were inoculated into Rapid Microbial Limit Test kits recommended for diagnostic microbiology supplied by Hi-media Laboratories Limited (Vignesh *et al*., 2014; 2015).

2.5 Heterotrophic bacterial studies

A total of sixty (60) bacterial strains were isolated from water and soil samples of the sampling sites. The serial dilution and pure culture techniques were used for isolation of bacterial strains and were used as test cultures (Vignesh *et al*., 2015). All the strains were isolated from the each location and were identified by the specific biochemical tests (Rapid Microbial Limit Test kits used) (Vignesh *et al*., 2014; Muthukumar *et al*., 2015).

2.6.Metal resistant studies

The test isolates were challenged against chromium metal (Potassium chromate) solutions with four different concentrations (10 mM, 50 mM, 100 mM and 250 mM) for metal resistant studies by plate diffusion and tube dilution methods. In plate diffusion assay, the 500 µL of chromium metal solution (four different concentration) was added to a central well (1 cm in diameter and 4 mm in depth) of nutrient agar plate separately and to allow it for metal diffusion at one day. In each metal concentration plate, eight bacterial isolates were inoculated in each plate by the radial streaking method. In tube dilution method (Minimal inhibitory concentration method), the appropriate volume of metal solution and 200 µL of standard culture (10^8 CFU/mL) were added into nutrient broth medium and make up into 10 mL with sterile nutrient broth. The test plates and tubes incubated at 37±1 °C for 48 h (Hassen *et al*., 1998). All the trials were performed in triplicate

2.7.Biosorption of Cu and Cr by dry biomass study

The bacterial strains were cultivated aerobically in 1000 ml conical flasks containing sterile nutrient broth on a rotary shaker (150 rpm) at 35º ± 2º C, separately. Cells were harvested at the end of exponential phase (after 48 h incubation) and for inactivation of the cells, the cultures were autoclaved (121º C, 15 min) before being harvested by centrifugation (10000 rpm for 20 min at room temperature) and finally freeze dried. The 10 mg dried cell of each biomass were mixed 10 ml of the metal solutions (Cu and Cr) in a two different flask separately. This process were carried with two different pH (pH – 4 and pH – 7) levels. The first and second flasks were agitated on a shaker (150 rpm) at 35º ± 2º C for 15 minutes and 2 h, respectively. All pH adjustment were made using reagent grade HCl and NaOH. After the treatment time, the samples were centrifuged at 10000 rpm for 20 min at room temperature and supernatant liquid was used to estimate metal ion concentrations (GBC SensAA - AAS, Australia - Flame mode) (De et al., 2007). The biosorption experiments were repeated three times and the mean values were reported.

2.8.Biosorption of Cu and Cr by heterotrophic bacterial study

The 5 mL of both 24 h cultures (bacterial strain 1 and 2) were inoculated into a 250 mL flask containing 150 mL of nutrient broth (pH - 7.0; Temperature - 35º ± 2º C; 150 rpm) supplemented with 50 ppm of copper. The inoculated flasks were incubated at 35º ± 2º C on a rotatory shaker (150 rpm) for 72 h. The same both cultures were used as a biosorbents in Cr treated process. The Cu and Cr removal was determined by analyzing the metal content of the medium. In 36 and 72 h, 5 mL of culture was withdrawn aseptically into a micro centrifuge tube and centrifuged at 10000 rpm for 15 min at 4º C. The collected supernatant was filtered through 0.22 µm pre-weighed nitro cellulose membrane filters and the filtrate was digested with 10% HNO_3 to estimate the copper and chromium in the supernatant All experiments were performed in triplicate and the mean value were reported. The metal removal rate was calculated using following formula.

$$\% \text{ Metal adsorbed} = (C_i - C_f) / C_i \quad \times 100$$

Where, C_i and C_f are the initial and equilibrium metal ion concentrations (mg L^{-1}), respectively.

3.RESULT.;

3.1i) Physiochemical studies

In oil contaminated water sample of post monsoon 2015, the mean values of pH, TDS, EC, DO, BOD, COD, TA, TH, Ca, Mg, Na, K, HCO_3, CO_3, Cl, SO_4, N-NO_2, O-PO_4 and oil/Greece were 7.6, 342.6, 580.7 (µS/cm), 6.3, 7.4, 11.3, 90.3, 81.9, 37.5, 44.4, 29.1, 20.6, 82.7, 0, 62.6, 47.2, 4.5, 4.7 and 6.5 mg/L, respectively..

3.2Heavy metal studies

In soil sample of post monsoon 2015, the mean values of Cd, Cr, Cu, Fe, Ni, Pb and Zn were 0.19, 0.10, 0.19, 1.07, 0.08, 0.11 and 0.62 mg/g, respectively. But in water sample of summer 2015, the range of Cd, Cr, Cu, Fe, Ni, Pb and Zn were 0.08 – 0.26, 0.06 – 0.12, 0.10 – 0.28, 0.41 – 1.57, BDL – 0.12, 0.06 – 0.12 and 0.31 – 1.08 mg/L, respectively.

3.3Microbiological studies

In water sample of post monsoon 2015, counts of TVC, TC, TS, FC, FS, VC, SAC, SHC and PC were in the range of 143000 – 91000, 1060 – 8400, 160 – 620, 210 – 910, 70 – 150, 80 – 130, 90 – 150 and 150 – 320 CFU/mL, respectively.

3.4 Multi-metal resistant studies

In chromium resistant study, a growth rate between 90-100% was observed for 86.5 % of the bacterial populations at 10 mM of Cr, whereas no population was growing at a growth rate of 0–80 % with 10 mM of Cr. At 50 mM of Cr, 53.5 % of the populations showed a growth rate of 81-90 % while 10 % of the population showed a growth rate of 0-80 %. were observed with a 71-80 % growth rate.

A growth rate between 91 - 100 % was observed for 91.5 % of the bacterial populations at 10 mM of Cu, whereas 16.5 % of population was growing at a growth rate of 0 – 80 % with 50 mM of Cu.

3.5.Bio sorption studies

In dry biomass study, at 5 ppm Cu treatment process, 57.6 % of Cu was observed by bacterial strain 1 in the pH 4 medium for 15 minutes time where as 62.4 % of Cu was observed at 2 h time.

In living cell study, copper removal by bacterial strain 1 were 27.6 and 36.4 % after 36 and 72 h of incubation, respectively. Chromium removal by bacterial strain 2 were 17.2 and 22.4 %, after 36 and 72 h of incubation, respectively. The dry biomass was act as an effective biosorbents than the living bio sorbents.

1Physiochemical parameters in oil contaminated regions, Tiruchirappalli – March to May 2015 (Summer

S.No	Sampling stations	Sample type	Sample name / nature	Sample code	Physiochemical parameters (mg/l or ppm = water; mg/kg = Soil)																		
					pH	TDS mg/l	EC µS/cm	Salinity ppt	DO mg/l	BOD mg/L	COD mg/L	TA mg/L	TH mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	K^{+} mg/L	HCO_3^{-} mg/L	CO_3^{-} mg/L	Cl^{-} mg/L	SO_4^{2-} mg/L	$N\text{-}NO^{2-}$ mg/L	$O\text{-}PO_4$ mg/L	Oil - Gre mg/L
1.	PRS	Water	OCW	W1	8.2	635.8	1077.6	1	4.8	6.6	15.5	153.8	150.1	71.6	78.5	52.4	41.5	142.8	0	124.3	94.5	10.5	8.5	12.8
2.	SOS	Water	OCW	W2	7.6	452.3	766.6	--	5.7	7.2	13.4	120.5	111.9	48.5	63.4	41.6	24.6	114.2	0	72.6	62.8	7.2	6.2	9.2
3.	CHB	Water	OCW	W3	7.8	247.7	419.8	--	6.5	8.4	9.5	61.4	52.9	21.5	31.4	19.8	15.4	58.5	0	51.8	32.6	2.1	3.4	4.8
4.	CLB	Water	OCW	W4	8.1	347	588.1	--	4.9	6.7	11.2	78.8	87.3	37.8	49.5	27.3	21.4	76.4	0	65.2	48.5	4.6	5.1	6.5
	Sum				*31.7*	*1682.8*	*2852.2*	*1.0*	*21.9*	*28.9*	*49.6*	*414.5*	*402.2*	*179.4*	*222.8*	*141.1*	*102.9*	*391.9*	*0.0*	*313.9*	*238.4*	*24.4*	*23.2*	*33.3*
	Average				*7.9*	*420.7*	*713.1*	*1.0*	*5.5*	*7.2*	*12.4*	*103.6*	*100.6*	*44.9*	*55.7*	*35.3*	*25.7*	*98.0*	*0.0*	*78.5*	*59.6*	*6.1*	*5.8*	*8.3*
	Maximum				*8.2*	*635.8*	*1077.6*	*1.0*	*6.5*	*8.4*	*15.5*	*153.8*	*150.1*	*71.6*	*78.5*	*52.4*	*41.5*	*142.8*	*0.0*	*124.3*	*94.5*	*10.5*	*8.5*	*12.8*
	Minimum				*7.6*	*247.7*	*419.8*	*1.0*	*4.8*	*6.6*	*9.5*	*61.4*	*52.9*	*21.5*	*31.4*	*19.8*	*15.4*	*58.5*	*0.0*	*51.8*	*32.6*	*2.1*	*3.4*	*4.8*
5.	PRS	Soil	OCS	S1	8.6	998.7	1692.7	1	7.1	8.2	19.8	201.4	200.4	115.8	84.6	116.5	54.2	192.5	0	290.4	112.6	11.2	9.7	17.5
6.	SOS	Soil	OCS	S2	8.2	636.4	1078.6	1	5.4	7.4	17.2	136.5	149.4	74.6	74.8	52.4	28.4	134.2	0	164.5	81.4	8.1	6.8	12.4
7.	CHB	Soil	OCS	S3	7.4	345	584.7	0	5.8	7.6	13.7	78.5	84.3	38.7	45.6	24.9	18.8	71.6	0	84.6	42.6	2.8	4.2	7.8
8.	CLB	Soil	OCS	S4	7.8	481.8	816.6	0	6.2	8.1	14.6	123.8	115.4	54.2	61.2	37.8	22.6	118.5	0	101.8	63.5	5.2	5.8	10.2
	Sum				*32*	*2461.9*	*4172.7*	*2*	*24.5*	*31.3*	*65.3*	*540.2*	*549.5*	*283.3*	*266.2*	*231.6*	*124.0*	*516.8*	*0.0*	*641.3*	*300.1*	*27.3*	*26.5*	*47.9*
	Average				*8*	*615.4*	*1043.2*	*0.5*	*6.12*	*7.82*	*16.32*	*135.05*	*137.37*	*70.82*	*66.55*	*57.9*	*31.0*	*129.2*	*0.0*	*160.3*	*75.0*	*6.8*	*6.6*	*12.0*
	Maximum				*8.6*	*998.7*	*1692.7*	*1*	*7.1*	*8.2*	*19.8*	*201.4*	*200.4*	*115.8*	*84.6*	*116.5*	*54.2*	*192.5*	*0.0*	*290.4*	*112.6*	*11.2*	*9.7*	*17.5*
	Minimum				*7.4*	*345*	*584.7*	*0*	*5.4*	*7.4*	*13.7*	*78.5*	*84.3*	*38.7*	*45.6*	*24.9*	*18.8*	*71.6*	*0.0*	*84.6*	*42.6*	*2.8*	*4.2*	*7.8*

PRS - Ponmalai Railway Shed; SOS – Senthaneerpuram Oil Shed; CHB – Chatram Bus Stand; CLB – Central Bus Stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TDS – Total dissolved solids; EC – Electrical conductivity; Salinity; DO – Dissolved oxygen; BOD – Biological dissolved oxygen; TA – Total alkalinity; TH – Total hardness; Ca – Calcium; Mg – Magnesium; Na – Sodium; K – Potassium; HCO_3 – Bicarbonate; CO_3 – Carbonate; Cl – Chloride; SO_4 – Sulphate; N-NO2 – Nitrite; $O\text{-}PO_4$ – Ortho-phosphate; Oil & Gre – Oil & Greece

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Table 4.2. Trace metal concentrations in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Trace metal parameters (mg/l or ppm = water; mg/kg = Soil)							Remarks	Reference
					Cd	Cr	Cu	Fe	Ni	Pb	Zn		
1.	PRS	Water	OCW	W1	0.17	0.1	0.19	1.06	0.08	0.12	0.82		
2.	SOS	Water	OCW	W2	0.11	0.08	0.15	0.39	0.06	0.1	0.41		
3.	CHB	Water	OCW	W3	0.08	BDL	0.1	0.22	0	0	0.2		
4.	CLB	Water	OCW	W4	0.08	BDL	0.14	0.35	0	0.08	0.25		
	Sum	*0.44*	*0.18*	*0.58*	*2.02*	*0.14*	*0.30*	*1.68*					
	Average	*0.11*	*0.09*	*0.15*	*0.51*	*0.04*	*0.08*	*0.42*					
	Maximum	*0.17*	*0.10*	*0.19*	*1.06*	*0.08*	*0.12*	*0.82*					
	Minimum	*0.08*	*0.08*	*0.10*	*0.22*	*0.00*	*0.00*	*0.20*					
5.	PRS	Soil	OCS	S1	0.28	0.12	0.24	1.75	0.12	0.11	0.84		
6.	SOS	Soil	OCS	S2	0.16	0.1	0.2	0.72	0.08	0.1	0.46		
7.	CHB	Soil	OCS	S3	0.12	0.08	0.1	0.33	0	0.08	0.21		
8.	CLB	Soil	OCS	S4	0.11	BDL	0.15	0.56	0.06	0.08	0.32		
	Sum	*0.67*	*0.30*	*0.69*	*3.36*	*0.26*	*0.37*	*1.83*					
	Average	*0.17*	*0.10*	*0.17*	*0.84*	*0.07*	*0.09*	*0.46*					
	Maximum	*0.28*	*0.12*	*0.24*	*1.75*	*0.12*	*0.11*	*0.84*					
	Minimum	*0.11*	*0.08*	*0.10*	*0.33*	*0.00*	*0.08*	*0.21*					

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

BDL – Below detectable limit (Not Determined); Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Ni – Nickel; Pb – Lead; Zn - Zinc

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Table 4.3 Microbiological levels/ counts in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Microbiological parameters (CFU/ml = water; CFU/g = Soil)									Remarks	Reference
					TVC	TC	TS	FC	FS	VC	SAC	SHC	PC		
Water															
1.	PRS	Water	OCW	W1	91000	8400	620	910	150	130	110	150	320		
2.	SOS	Water	OCW	W2	62000	3200	470	670	120	90	90	120	240		
3.	CHB	Water	OCW	W3	14300	1060	160	210	70	80	80	90	150		
4.	CLB	Water	OCW	W4	21600	1640	240	320	100	90	100	110	180		
	Sum				*188900*	*14300*	*1490*	*2110*	*440*	*390*	*380*	*470*	*890*		
	Average				*47225*	*3575*	*372.5*	*527.5*	*110*	*97.5*	*95*	*117.5*	*222.5*		
	Maximum				*91000*	*8400*	*620*	*910*	*150*	*130*	*110*	*150*	*320*		
	Minimum				*14300*	*1060*	*160*	*210*	*70*	*80*	*80*	*90*	*150*		
5.	PRS	Soil	OCS	S1	156000	10100	940	1030	220	150	130	190	640		
6.	SOS	Soil	OCS	S2	92000	4100	720	850	150	130	110	160	460		
7.	CHB	Soil	OCS	S3	38000	1920	200	260	90	90	90	100	240		
8.	CLB	Soil	OCS	S4	61000	3300	310	580	130	110	110	120	340		
	Sum				*347000*	*19420*	*2170*	*2720*	*590*	*480*	*440*	*570*	*1680*		
	Average				*86750*	*4855*	*542.5*	*680*	*147.5*	*120*	*110*	*142.5*	*420*		
	Maximum				*156000*	*10100*	*940*	*1030*	*220*	*150*	*130*	*190*	*640*		
	Minimum				*38000*	*1920*	*200*	*260*	*90*	*90*	*90*	*100*	*240*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TVC – Total viable count; TC – Total coliforms; TS – Total *Streptococci*; FC – Fecal coliforms; FS – Fecal *Streptococci*; VC – *Vibrio* count; SAC – *Salmonella* count; SHC – *Shigella* count; PC – *Pseudomonas* count;

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Table 4.4. Trace metal concentrations in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon

S.No	Sampling stations	Sample type	Sample name	Sample code	Trace metal parameters (mg/l or ppm = water; mg/kg = Soil)							Remarks	Reference
					Cd	Cr	Cu	Fe	Ni	Pb	Zn		
1.	PRS	Water	OCW	W1	0.22	0.1	0.21	1.24	0.1	0.1	0.94		
2.	SOS	Water	OCW	W2	0.12	0.08	0.13	0.48	0.08	0.08	0.54		
3.	CHB	Water	OCW	W3	0.06	0.08	0.08	0.25	0	0	0.26		
4.	CLB	Water	OCW	W4	0.08	0.08	0.12	0.42	0	0.08	0.35		
	Sum				*0.48*	*0.34*	*0.54*	*2.39*	*0.18*	*0.26*	*2.09*		
	Average				*0.12*	*0.09*	*0.14*	*0.60*	*0.05*	*0.07*	*0.52*		
	Maximum				*0.22*	*0.10*	*0.21*	*1.24*	*0.10*	*0.10*	*0.94*		
	Minimum				*0.06*	*0.08*	*0.08*	*0.25*	*0.00*	*0.00*	*0.26*		
5.	PRS	Soil	OCS	S1	0.35	0.15	0.31	2.14	0.14	0.15	1.12		
6.	SOS	Soil	OCS	S2	0.19	0.1	0.18	0.98	0.1	0.11	0.63		
7.	CHB	Soil	OCS	S3	0.1	0.06	0.12	0.48	0	0.08	0.3		
8.	CLB	Soil	OCS	S4	0.12	0.08	0.16	0.67	0.08	0.1	0.42		
	Sum				*0.76*	*0.39*	*0.77*	*4.27*	*0.32*	*0.44*	*2.47*		
	Average				*0.19*	*0.10*	*0.19*	*1.07*	*0.08*	*0.11*	*0.62*		
	Maximum				*0.35*	*0.15*	*0.31*	*2.14*	*0.14*	*0.15*	*1.12*		
	Minimum				*0.10*	*0.06*	*0.12*	*0.48*	*0.00*	*0.08*	*0.30*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

BDL – Below detectable limit (Not Determined); Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Ni – Nickel; Pb – Lead; Zn - Zinc

Amir H. Charkhabi, Mohamad Sakizadeh and Gholamreza Rafiee, (2005). Seasonal Fluctuation in Heavy Metal Pollution in Iran's Siahroud GW. Environ Sci & Pollut Res, 12 (5) 264 – 270.

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S. Dhanakumar, K. Rutharvel Murthy, G. Solaraj, R. Mohanraj, (2013). Heavy-Metal Fractionation in Surface Sediments of the Cauvery GW Estuarine Region, Southeastern Coast of India. Arch Environ Contam Toxicol, 65 (1), 14 – 23.

Table 4.5. Physiochemical parameters in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon)

S.No	Sampling stations	Sample type	Sample name / nature	Sample code	Physiochemical parameters (mg/l or ppm = water; mg/kg = Soil)																			
					pH	TDS mg/l	EC µS/cm	Salinity ppt	DO mg/l	BOD mg/L	COD mg/L	TA mg/L	TH mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	K^{+} mg/L	HCO_3^{-} mg/L	CO_3^{-} mg/L	Cl^{-} mg/L	SO_4^{2-} mg/L	$N\text{-}NO^{2-}$ mg/L	$O\text{-}PO_4$ mg/L	Oil - Gre mg/L
1.	PRS	Water	OCW	W1	7.8	529.4	897.3	--	6.4	7.2	14.2	132.5	124.9	59.5	65.4	42.5	32.8	121.8	0	106.5	76.5	8.2	6.8	9.4
2.	SOS	Water	OCW	W2	7.4	375.3	636.1	--	6.8	8.4	11.5	105.4	92.3	42.1	50.2	33.8	19.8	98.5	0	61.4	50.4	4.6	5.1	6.4
3.	CHB	Water	OCW	W3	8.1	182.3	309.0	--	6.2	7.6	8.5	49.8	42.1	17.5	24.6	16.4	11.5	42.5	0	33.6	22.4	1.8	2.6	3.8
4.	CLB	Water	OCW	W4	7.2	283.5	480.5	--	5.8	6.4	10.8	73.4	68.2	30.8	37.4	23.8	18.4	67.9	0	48.7	39.4	3.4	4.3	6.2
	Sum				*30.5*	*1370.5*	*2322.9*	*0.0*	*25.2*	*29.6*	*45.0*	*361.1*	*327.5*	*149.9*	*177.6*	*116.5*	*82.5*	*330.7*	*0.0*	*250.2*	*188.7*	*18.0*	*18.8*	*25.8*
	Average				*7.6*	*342.6*	*580.7*	*0.0*	*6.3*	*7.4*	*11.3*	*90.3*	*81.9*	*37.5*	*44.4*	*29.1*	*20.6*	*82.7*	*0.0*	*62.6*	*47.2*	*4.5*	*4.7*	*6.5*
	Maximum				*8.1*	*529.4*	*897.3*	*0.0*	*6.8*	*8.4*	*14.2*	*132.5*	*124.9*	*59.5*	*65.4*	*42.5*	*32.8*	*121.8*	*0.0*	*106.5*	*76.5*	*8.2*	*6.8*	*9.4*
	Minimum				*7.2*	*182.3*	*309.0*	*0.0*	*5.8*	*6.4*	*8.5*	*49.8*	*42.1*	*17.5*	*24.6*	*16.4*	*11.5*	*42.5*	*0.0*	*33.6*	*22.4*	*1.8*	*2.6*	*3.8*
5.	PRS	Soil	OCS	S1	8.2	814.5	1380.5	1	6.4	7.8	16.5	168.4	175.1	98.7	76.4	92.4	44.8	162.8	0	212.5	98.5	10.5	8.5	14.6
6.	SOS	Soil	OCS	S2	7.9	531.9	901.5	0	5.8	7.1	13.4	124.3	121.6	60.2	61.4	45.6	24.3	118.7	0	126.8	72.6	6.7	6.2	10.5
7.	CHB	Soil	OCS	S3	6.8	266.8	452.2	0	6.5	8.2	10.8	62.5	63	29.4	33.6	20.8	15.4	60.5	0	61.7	30.4	2.2	3.4	6.8
8.	CLB	Soil	OCS	S4	7.5	392.5	665.3	0	6.9	8.4	12.6	103.4	94.4	41.6	52.8	30.5	20.8	99.4	0	74.6	53.8	4.4	5.2	8.7
	Sum				*30.4*	*2005.7*	*3399.5*	*1*	*25.6*	*31.5*	*53.3*	*458.6*	*454.1*	*229.9*	*224.2*	*189.3*	*105.3*	*441.4*	*0.0*	*475.6*	*255.3*	*23.8*	*23.3*	*40.6*
	Average				*7.6*	*501.42*	*849.9*	*0.25*	*6.4*	*7.87*	*13.32*	*114.65*	*113.52*	*57.475*	*56.05*	*47.3*	*26.3*	*110.4*	*0.0*	*118.9*	*63.8*	*6.0*	*5.8*	*10.2*
	Maximum				*8.2*	*814.5*	*1380.5*	*1*	*6.9*	*8.4*	*16.5*	*168.4*	*175.1*	*98.7*	*76.4*	*92.4*	*44.8*	*162.8*	*0.0*	*212.5*	*98.5*	*10.5*	*8.5*	*14.6*
	Minimum				*6.8*	*266.8*	*452.2*	*0*	*5.8*	*7.1*	*10.8*	*62.5*	*63*	*29.4*	*33.6*	*20.8*	*15.4*	*60.5*	*0.0*	*61.7*	*30.4*	*2.2*	*3.4*	*6.8*

PRS - Ponmalai Railway Shed; SOS – Senthaneerpuram Oil Shed; CHB – Chatram Bus Stand; CLB – Central Bus Stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TDS – Total dissolved solids; EC – Electrical conductivity; Salinity; DO – Dissolved oxygen; BOD – Biological dissolved oxygen; TA – Total alkalinity; TH – Total hardness; Ca – Calcium; Mg – Magnesium; Na – Sodium; K – Potassium; HCO_3 – Bicarbonate; CO_3 – Carbonate; Cl – Chloride; SO_4 – Sulphate; N-NO2 – Nitrite; $O\text{-}PO_4$ – Ortho-phosphate; Oil & Gre – Oil & Greece APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. 19th edn, Washington, DC.

Vignesh, S., Dahms, HU., Emmanuel, KV., Gokul, MS., Muthukumar, K., Kim, BR., James, RA. (2014). Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India, Environ monit and assess, 186 (3), 1875 – 1887.

Table 4.6 Trace metal concentrations in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Trace metal parameters (mg/l or ppm = water; mg/kg = Soil)							Remarks	Reference
					Cd	Cr	Cu	Fe	Ni	Pb	Zn		
1.	PRS	Water	OCW	W1	0.22	0.1	0.21	1.24	0.1	0.1	0.94		
2.	SOS	Water	OCW	W2	0.12	0.08	0.13	0.48	0.08	0.08	0.54		
3.	CHB	Water	OCW	W3	0.06	0.08	0.08	0.25	0	0	0.26		
4.	CLB	Water	OCW	W4	0.08	0.08	0.12	0.42	0	0.08	0.35		
	Sum				*0.48*	*0.34*	*0.54*	*2.39*	*0.18*	*0.26*	*2.09*		
	Average				*0.12*	*0.09*	*0.14*	*0.60*	*0.05*	*0.07*	*0.52*		
	Maximum				*0.22*	*0.10*	*0.21*	*1.24*	*0.10*	*0.10*	*0.94*		
	Minimum				*0.06*	*0.08*	*0.08*	*0.25*	*0.00*	*0.00*	*0.26*		
5.	PRS	Soil	OCS	S1	0.35	0.15	0.31	2.14	0.14	0.15	1.12		
6.	SOS	Soil	OCS	S2	0.19	0.1	0.18	0.98	0.1	0.11	0.63		
7.	CHB	Soil	OCS	S3	0.1	0.06	0.12	0.48	0	0.08	0.3		
8.	CLB	Soil	OCS	S4	0.12	0.08	0.16	0.67	0.08	0.1	0.42		
	Sum				*0.76*	*0.39*	*0.77*	*4.27*	*0.32*	*0.44*	*2.47*		
	Average				*0.19*	*0.10*	*0.19*	*1.07*	*0.08*	*0.11*	*0.62*		
	Maximum				*0.35*	*0.15*	*0.31*	*2.14*	*0.14*	*0.15*	*1.12*		
	Minimum				*0.10*	*0.06*	*0.12*	*0.48*	*0.00*	*0.08*	*0.30*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

BDL – Below detectable limit (Not Determined); Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Ni – Nickel; Pb – Lead; Zn - Zinc

Amir H. Charkhabi, Mohamad Sakizadeh and Gholamreza Rafiee, (2005). Seasonal Fluctuation in Heavy Metal Pollution in Iran's Siahroud GW. Environ Sci & Pollut Res, 12 (5) 264 – 270.

N. Pourang, A. Nikouyan and J. H. Dennis, (2005). Trace element concentrations in fish, surficial sediments and water from northern part of the Persian gulf. Environmental Monitoring and Assessment, 109: 293–316.

S. Dhanakumar, K. Rutharvel Murthy, G. Solaraj, R. Mohanraj, (2013). Heavy-Metal Fractionation in Surface Sediments of the Cauvery GW Estuarine Region, Southeastern Coast of India. Arch Environ Contam Toxicol, 65 (1), 14 – 23.

Table 4.7 Microbiological levels/ counts in oil contaminated regions, Tiruchirappalli – January to February 2015 (Post monsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Microbiological parameters (CFU/ml = water; CFU/g = Soil)									Remarks	Reference
					TVC	TC	TS	FC	FS	VC	SAC	SHC	PC		
Water															
1.	PRS	Water	OCW	W1	91000	8400	620	910	150	130	110	150	320		
2.	SOS	Water	OCW	W2	62000	3200	470	670	120	90	90	120	240		
3.	CHB	Water	OCW	W3	14300	1060	160	210	70	80	80	90	150		
4.	CLB	Water	OCW	W4	21600	1640	240	320	100	90	100	110	180		
	Sum				*188900*	*14300*	*1490*	*2110*	*440*	*390*	*380*	*470*	*890*		
	Average				*47225*	*3575*	*372.5*	*527.5*	*110*	*97.5*	*95*	*117.5*	*222.5*		
	Maximum				*91000*	*8400*	*620*	*910*	*150*	*130*	*110*	*150*	*320*		
	Minimum				*14300*	*1060*	*160*	*210*	*70*	*80*	*80*	*90*	*150*		
5.	PRS	Soil	OCS	S1	156000	10100	940	1030	220	150	130	190	640		
6.	SOS	Soil	OCS	S2	92000	4100	720	850	150	130	110	160	460		
7.	CHB	Soil	OCS	S3	38000	1920	200	260	90	90	90	100	240		
8.	CLB	Soil	OCS	S4	61000	3300	310	580	130	110	110	120	340		
	Sum				*347000*	*19420*	*2170*	*2720*	*590*	*480*	*440*	*570*	*1680*		
	Average				*86750*	*4855*	*542.5*	*680*	*147.5*	*120*	*110*	*142.5*	*420*		
	Maximum				*156000*	*10100*	*940*	*1030*	*220*	*150*	*130*	*190*	*640*		
	Minimum				*38000*	*1920*	*200*	*260*	*90*	*90*	*90*	*100*	*240*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TVC – Total viable count; TC – Total coliforms; TS – Total *Streptococci*; FC – Fecal coliforms; FS – Fecal *Streptococci*; VC – *Vibrio* count; SAC – *Salmonella* count; SHC – *Shigella* count; PC – *Pseudomonas* count;

Clark A, Turner T, Dorothy KP, Goutham J, Kalavati C, Rajanna B (2003) Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. Ecotoxicology and Environmental Safety 56: 390–397. doi: 10.1016/S0147-6513(03)00098-8. Pubmed: 14575679.

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Table 4.8. Physiochemical parameters in oil contaminated regions, Tiruchirappalli – June to August 2015 (Premonsoon)

S.No	Sampling stations	Sample type	Sample name / nature	Sample code	Physiochemical parameters (mg/l or ppm = water; mg/kg = Soil)																		
					pH	TDS mg/l	EC μS/cm	Salinity ppt	DO mg/l	BOD mg/L	COD mg/L	TA mg/L	TH mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	K^{+} mg/L	HCO_3^{-} mg/L	CO_3^{-} mg/L	Cl^{-} mg/L	SO_4^{2-} mg/L	$N\text{-}NO^{2-}$ mg/L	$O\text{-}PO_4$ mg/L	Oil - Gre mg/L
1.	PRS	Water	OCW	W1	8.2	439.8	745.4	0	5.5	6.4	12.5	110.2	104.6	47.2	57.4	34.6	26.4	102.8	0	84.5	64.5	7.1	5.9	7.4
2.	SOS	Water	OCW	W2	7.6	309.3	524.2	0	6.2	7.4	10.2	84.6	78	38.5	39.5	25.8	17.2	80.4	0	52.4	38.4	3.5	4.2	5.2
3.	CHB	Water	OCW	W3	7.9	140.2	237.6	0	5.7	6.9	7.4	35.8	32.6	14.2	18.4	12.4	9.5	30.6	0	23.8	18.4	1.5	2	3
4.	CLB	Water	OCW	W4	7.5	227.8	386.1	0	6.3	7.1	9.5	58.4	54.2	24.6	29.6	20.9	14.8	52.5	0	40.3	30.2	2.4	3.1	4.8
	Sum				31.2	1117.1	1893.4	0.0	23.7	27.8	39.6	289.0	269.4	124.5	144.9	93.7	67.9	266.3	0.0	201.0	151.5	14.5	15.2	20.4
	Average				7.8	279.3	473.3	0.0	5.9	7.0	9.9	72.3	67.4	31.1	36.2	23.4	17.0	66.6	0.0	50.3	37.9	3.6	3.8	5.1
	Maximum				8.2	439.8	745.4	0.0	6.3	7.4	12.5	110.2	104.6	47.2	57.4	34.6	26.4	102.8	0.0	84.5	64.5	7.1	5.9	7.4
	Minimum				7.5	140.2	237.6	0.0	5.5	6.4	7.4	35.8	32.6	14.2	18.4	12.4	9.5	30.6	0.0	23.8	18.4	1.5	2.0	3.0
5.	PRS	Soil	OCS	S1	7.9	675.6	1145.1	1	6.8	7.6	13.2	140.2	138.2	79.8	58.4	80.4	35.4	132.4	0	184.2	80.6	8.2	6.8	10.5
6.	SOS	Soil	OCS	S2	7.5	425.6	721.4	0	5.5	7.4	11.8	99.5	91.7	46.5	45.2	31.6	20.1	95.4	0	108.5	58.4	5.4	5.1	7.4
7.	CHB	Soil	OCS	S3	8.2	211.9	359.2	0	7.2	8.6	8.4	51.2	49.8	23.4	26.4	15.4	12.6	43.5	0	53.4	23.4	1.8	2.6	4.1
8.	CLB	Soil	OCS	S4	7.4	317.7	538.5	0	7.6	8.4	10.6	86.4	77.3	36.8	40.5	26.4	16.4	78.2	0	60.6	41.5	3.4	4.5	6.5
	Sum				31	1630.8	2764.1	1.0	27.1	32.0	44.0	377.3	357.0	186.5	170.5	153.8	84.5	349.5	0.0	406.7	203.9	18.8	19.0	28.5
	Average				7.75	407.7	691.0	0.3	6.8	8.0	11.0	94.3	89.3	46.6	42.6	38.5	21.1	87.4	0.0	101.7	51.0	4.7	4.8	7.1
	Maximum				8.2	675.6	1145.1	1.0	7.6	8.6	13.2	140.2	138.2	79.8	58.4	80.4	35.4	132.4	0.0	184.2	80.6	8.2	6.8	10.5
	Minimum				7.4	211.9	359.2	0.0	5.5	7.4	8.4	51.2	49.8	23.4	26.4	15.4	12.6	43.5	0.0	53.4	23.4	1.8	2.6	4.1

PRS - Ponmalai Railway Shed; SOS – Senthaneerpuram Oil Shed; CHB – Chatram Bus Stand; CLB – Central Bus Stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TDS – Total dissolved solids; EC – Electrical conductivity; Salinity; DO – Dissolved oxygen; BOD – Biological dissolved oxygen; TA – Total alkalinity; TH – Total hardness; Ca – Calcium; Mg – Magnesium; Na – Sodium; K – Potassium; HCO_3 – Bicarbonate; CO_3 – Carbonate; Cl – Chloride; SO_4 – Sulphate; N-NO2 – Nitrite; $O\text{-}PO_4$ – Ortho-phosphate; Oil & Gre – Oil & Greece

APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. 19th edn, Washington, DC.

Vignesh, S., Dahms, HU., Emmanuel, KV., Gokul, MS., Muthukumar, K., Kim, BR., James, RA. (2014). Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India, Environ monit and assess, 186 (3), 1875 – 1887.

Table 4.9. Trace metal concentrations in oil contaminated regions, Tiruchirappalli – June to August 2015 (Premonsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Trace metal parameters (mg/l or ppm = water; mg/kg = Soil)							Remarks	Reference
					Cd	Cr	Cu	Fe	Ni	Pb	Zn		
1.	PRS	Water	OCW	W1	0.17	0.1	0.19	1.06	0.08	0.12	0.82		
2.	SOS	Water	OCW	W2	0.11	0.08	0.15	0.39	0.06	0.1	0.41		
3.	CHB	Water	OCW	W3	0.08	BDL	0.1	0.22	0	0	0.2		
4.	CLB	Water	OCW	W4	0.08	BDL	0.14	0.35	0	0.08	0.25		
	Sum				*0.44*	*0.18*	*0.58*	*2.02*	*0.14*	*0.30*	*1.68*		
	Average				*0.11*	*0.09*	*0.15*	*0.51*	*0.04*	*0.08*	*0.42*		
	Maximum				*0.17*	*0.10*	*0.19*	*1.06*	*0.08*	*0.12*	*0.82*		
	Minimum				*0.08*	*0.08*	*0.10*	*0.22*	*0.00*	*0.00*	*0.20*		
5.	PRS	Soil	OCS	S1	0.28	0.12	0.24	1.75	0.12	0.11	0.84		
6.	SOS	Soil	OCS	S2	0.16	0.1	0.2	0.72	0.08	0.1	0.46		
7.	CHB	Soil	OCS	S3	0.12	0.08	0.1	0.33	0	0.08	0.21		
8.	CLB	Soil	OCS	S4	0.11	BDL	0.15	0.56	0.06	0.08	0.32		
	Sum				*0.67*	*0.30*	*0.69*	*3.36*	*0.26*	*0.37*	*1.83*		
	Average				*0.17*	*0.10*	*0.17*	*0.84*	*0.07*	*0.09*	*0.46*		
	Maximum				*0.28*	*0.12*	*0.24*	*1.75*	*0.12*	*0.11*	*0.84*		
	Minimum				*0.11*	*0.08*	*0.10*	*0.33*	*0.00*	*0.08*	*0.21*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

BDL – Below detectable limit (Not Determined); Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Ni – Nickel; Pb – Lead; Zn - Zinc

Amir H. Charkhabi, Mohamad Sakizadeh and Gholamreza Rafiee, (2005). Seasonal Fluctuation in Heavy Metal Pollution in Iran's Siahroud GW. Environ Sci & Pollut Res, 12 (5) 264 – 270.

N. Pourang, A. Nikouyan and J. H. Dennis, (2005). Trace element concentrations in fish, surficial sediments and water from northern part of the Persian gulf. Environmental Monitoring and Assessment, 109: 293–316.

S. Dhanakumar, K. Rutharvel Murthy, G. Solaraj, R. Mohanraj, (2013). Heavy-Metal Fractionation in Surface Sediments of the Cauvery GW Estuarine Region, Southeastern Coast of India. Arch Environ Contam Toxicol, 65 (1), 14 – 23.

Table 4. 10. Microbiological levels/ counts in oil contaminated regions, Tiruchirappalli – June to August 2015 (Premonsoon)

S.No	Sampling stations	Sample type	Sample name	Sample code	Microbiological parameters (CFU/ml = water; CFU/g = Soil)									Remarks	Reference
					TVC	TC	TS	FC	FS	VC	SAC	SHC	PC		
Water															
1.	PRS	Water	OCW	W1	74000	5200	430	620	120	130	90	140	260		
2.	SOS	Water	OCW	W2	46000	2130	310	430	100	100	50	100	150		
3.	CHB	Water	OCW	W3	11600	940	140	150	60	110	60	70	100		
4.	CLB	Water	OCW	W4	18200	1350	200	240	90	80	80	90	140		
	Sum				*149800*	*9620*	*1080*	*1440*	*370*	*420*	*280*	*400*	*650*		
	Average				*37450*	*2405*	*270*	*360*	*92.5*	*105*	*70*	*100*	*162.5*		
	Maximum				*74000*	*5200*	*430*	*620*	*120*	*130*	*90*	*140*	*260*		
	Minimum				*11600*	*940*	*140*	*150*	*60*	*80*	*50*	*70*	*100*		
5.	PRS	Soil	OCS	S1	129000	8700	710	810	170	130	120	160	460		
6.	SOS	Soil	OCS	S2	76000	3300	540	620	120	100	90	120	320		
7.	CHB	Soil	OCS	S3	30000	1540	160	210	70	70	60	90	200		
8.	CLB	Soil	OCS	S4	49000	2300	250	370	100	90	100	110	260		
	Sum				*284000*	*15840*	*1660*	*2010*	*460*	*390*	*370*	*480*	*1240*		
	Average				*71000*	*3960*	*415*	*502.5*	*115*	*97.5*	*92.5*	*120*	*310*		
	Maximum				*129000*	*8700*	*710*	*810*	*170*	*130*	*120*	*160*	*460*		
	Minimum				*30000*	*1540*	*160*	*210*	*70*	*70*	*60*	*90*	*200*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TVC – Total viable count; TC – Total coliforms; TS – Total *Streptococci*; FC – Fecal coliforms; FS – Fecal *Streptococci*; VC – *Vibrio* count; SAC – *Salmonella* count; SHC – *Shigella* count; PC – *Pseudomonas* count;

Clark A, Turner T, Dorothy KP, Goutham J, Kalavati C, Rajanna B (2003) Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. Ecotoxicology and Environmental Safety 56: 390–397. doi: 10.1016/S0147-6513(03)00098-8. Pubmed: 14575679.

Vignesh S, Muthukumar K, James RA (2012) Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. Marine Pollution Bulletin 64: 790–800. doi: 10.1016/j.marpolbul.2012.01.015. Pubmed: 22321173.

Vignesh S, Dahms HU, Emmanuel KV, Gokul MS, Muthukumar K, Kim BR, James RA (2014) Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India. Environmental Monitoring and Assessment 186(3): 1875–1887. doi: 10.1007/s10661-013-3501-z. Pubmed: 24292984.

Table 4. 11. Physiochemical parameters in oil contaminated regions, Tiruchirappalli – March to May 2015 (Summer)

S.No	Sampling stations	Sample type	Sample name / nature	Sample code	Physiochemical parameters (mg/l or ppm = water; mg/kg = Soil)																		
					pH	TDS mg/l	EC μS/cm	Salinity ppt	DO mg/l	BOD mg/L	COD mg/L	TA mg/L	TH mg/L	Ca^{2+} mg/L	Mg^{2+} mg/L	Na^{+} mg/L	K^{+} mg/L	HCO_3^{-} mg/L	CO_3^{-} mg/L	Cl^{-} mg/L	SO_4^{2-} mg/L	$N\text{-}NO^{2-}$ mg/L	$O\text{-}PO_4$ mg/L	Oil - Gre mg/L
1.	PRS	Water	OCW	W1	8.2	635.8	1077.6	1	4.8	6.6	15.5	153.8	150.1	71.6	78.5	52.4	41.5	142.8	0	124.3	94.5	10.5	8.5	12.8
2.	SOS	Water	OCW	W2	7.6	452.3	766.6	--	5.7	7.2	13.4	120.5	111.9	48.5	63.4	41.6	24.6	114.2	0	72.6	62.8	7.2	6.2	9.2
3.	CHB	Water	OCW	W3	7.8	247.7	419.8	--	6.5	8.4	9.5	61.4	52.9	21.5	31.4	19.8	15.4	58.5	0	51.8	32.6	2.1	3.4	4.8
4.	CLB	Water	OCW	W4	8.1	347	588.1	--	4.9	6.7	11.2	78.8	87.3	37.8	49.5	27.3	21.4	76.4	0	65.2	48.5	4.6	5.1	6.5
	Sum				*31.7*	*1682.8*	*2852.2*	*1.0*	*21.9*	*28.9*	*49.6*	*414.5*	*402.2*	*179.4*	*222.8*	*141.1*	*102.9*	*391.9*	*0.0*	*313.9*	*238.4*	*24.4*	*23.2*	*33.3*
	Average				*7.9*	*420.7*	*713.1*	*1.0*	*5.5*	*7.2*	*12.4*	*103.6*	*100.6*	*44.9*	*55.7*	*35.3*	*25.7*	*98.0*	*0.0*	*78.5*	*59.6*	*6.1*	*5.8*	*8.3*
	Maximum				*8.2*	*635.8*	*1077.6*	*1.0*	*6.5*	*8.4*	*15.5*	*153.8*	*150.1*	*71.6*	*78.5*	*52.4*	*41.5*	*142.8*	*0.0*	*124.3*	*94.5*	*10.5*	*8.5*	*12.8*
	Minimum				*7.6*	*247.7*	*419.8*	*1.0*	*4.8*	*6.6*	*9.5*	*61.4*	*52.9*	*21.5*	*31.4*	*19.8*	*15.4*	*58.5*	*0.0*	*51.8*	*32.6*	*2.1*	*3.4*	*4.8*
5.	PRS	Soil	OCS	S1	8.6	998.7	1692.7	1	7.1	8.2	19.8	201.4	200.4	115.8	84.6	116.5	54.2	192.5	0	290.4	112.6	11.2	9.7	17.5
6.	SOS	Soil	OCS	S2	8.2	636.4	1078.6	1	5.4	7.4	17.2	136.5	149.4	74.6	74.8	52.4	28.4	134.2	0	164.5	81.4	8.1	6.8	12.4
7.	CHB	Soil	OCS	S3	7.4	345	584.7	0	5.8	7.6	13.7	78.5	84.3	38.7	45.6	24.9	18.8	71.6	0	84.6	42.6	2.8	4.2	7.8
8.	CLB	Soil	OCS	S4	7.8	481.8	816.6	0	6.2	8.1	14.6	123.8	115.4	54.2	61.2	37.8	22.6	118.5	0	101.8	63.5	5.2	5.8	10.2
	Sum				*32*	*2461.9*	*4172.7*	*2*	*24.5*	*31.3*	*65.3*	*540.2*	*549.5*	*283.3*	*266.2*	*231.6*	*124.0*	*516.8*	*0.0*	*641.3*	*300.1*	*27.3*	*26.5*	*47.9*
	Average				*8*	*615.4*	*1043.2*	*0.5*	*6.12*	*7.82*	*16.32*	*135.05*	*137.37*	*70.82*	*66.55*	*57.9*	*31.0*	*129.2*	*0.0*	*160.3*	*75.0*	*6.8*	*6.6*	*12.0*
	Maximum				*8.6*	*998.7*	*1692.7*	*1*	*7.1*	*8.2*	*19.8*	*201.4*	*200.4*	*115.8*	*84.6*	*116.5*	*54.2*	*192.5*	*0.0*	*290.4*	*112.6*	*11.2*	*9.7*	*17.5*
	Minimum				*7.4*	*345*	*584.7*	*0*	*5.4*	*7.4*	*13.7*	*78.5*	*84.3*	*38.7*	*45.6*	*24.9*	*18.8*	*71.6*	*0.0*	*84.6*	*42.6*	*2.8*	*4.2*	*7.8*

PRS - Ponmalai Railway Shed; SOS – Senthaneerpuram Oil Shed; CHB – Chatram Bus Stand; CLB – Central Bus Stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TDS – Total dissolved solids; EC – Electrical conductivity; Salinity; DO – Dissolved oxygen; BOD – Biological dissolved oxygen; TA – Total alkalinity; TH – Total hardness; Ca – Calcium; Mg – Magnesium; Na – Sodium; K – Potassium; HCO_3 – Bicarbonate; CO_3 – Carbonate; Cl – Chloride; SO_4 – Sulphate; N-NO2 – Nitrite; $O\text{-}PO_4$ – Ortho-phosphate; Oil & Gre – Oil & Greece

APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. 19th edn, Washington, DC.

Vignesh, S., Dahms, HU., Emmanuel, KV., Gokul, MS., Muthukumar, K., Kim, BR., James, RA. (2014). Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India, Environ monit and assess, 186 (3), 1875 – 1887.

Table 4. 12. Trace metal concentrations in oil contaminated regions, Tiruchirappalli – March to May 2015 (Summer)

S.No	Sampling stations	Sample type	Sample name	Sample code	Trace metal parameters (mg/l or ppm = water; mg/kg = Soil)							Remarks	Reference
					Cd	Cr	Cu	Fe	Ni	Pb	Zn		
1.	PRS	Water	OCW	W1	0.26	0.12	0.28	1.57	0.12	0.12	1.08		
2.	SOS	Water	OCW	W2	0.15	0.08	0.16	0.64	0.1	0.11	0.62		
3.	CHB	Water	OCW	W3	0.08	0.06	0.1	0.41	BDL	0.06	0.31		
4.	CLB	Water	OCW	W4	0.1	0.1	0.13	0.58	0.08	0.08	0.48		
	Sum				*0.59*	*0.36*	*0.67*	*3.20*	*0.30*	*0.37*	*2.49*		
	Average				*0.15*	*0.09*	*0.17*	*0.80*	*0.10*	*0.09*	*0.62*		
	Maximum				*0.26*	*0.12*	*0.28*	*1.57*	*0.12*	*0.12*	*1.08*		
	Minimum				*0.08*	*0.06*	*0.10*	*0.41*	*0.08*	*0.06*	*0.31*		
5.	PRS	Soil	OCS	S1	0.48	0.18	0.42	2.84	0.16	0.19	1.42		
6.	SOS	Soil	OCS	S2	0.25	0.12	0.25	1.24	0.12	0.14	0.75		
7.	CHB	Soil	OCS	S3	0.12	0.01	0.14	0.65	BDL	0.1	0.44		
8.	CLB	Soil	OCS	S4	0.16	0.11	0.17	0.97	0.1	0.12	0.61		
	Sum				*1.01*	*0.42*	*0.98*	*5.70*	*0.38*	*0.55*	*3.22*		
	Average				*0.25*	*0.11*	*0.25*	*1.43*	*0.13*	*0.14*	*0.81*		
	Maximum				*0.48*	*0.18*	*0.42*	*2.84*	*0.16*	*0.19*	*1.42*		
	Minimum				*0.12*	*0.01*	*0.14*	*0.65*	*0.10*	*0.10*	*0.44*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

~ = Approximately; OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

BDL – Below detectable limit (Not Determined); Cd – Cadmium; Cr – Chromium; Cu – Copper; Fe – Iron; Ni – Nickel; Pb – Lead; Zn - Zinc

Amir H. Charkhabi, Mohamad Sakizadeh and Gholamreza Rafiee, (2005). Seasonal Fluctuation in Heavy Metal Pollution in Iran's Siahroud GW. Environ Sci & Pollut Res, 12 (5) 264 – 270.

N. Pourang, A. Nikouyan and J. H. Dennis, (2005). Trace element concentrations in fish, surficial sediments and water from northern part of the Persian gulf. Environmental Monitoring and Assessment, 109: 293–316.

S. Dhanakumar, K. Rutharvel Murthy, G. Solaraj, R. Mohanraj, (2013). Heavy-Metal Fractionation in Surface Sediments of the Cauvery GW Estuarine Region, Southeastern Coast of India. Arch Environ Contam Toxicol, 65 (1), 14 – 23.

Table 4. 13. Microbiological levels/ counts in oil contaminated regions, Tiruchirappalli – March to May 2015 (Summer)

S.No	Sampling stations	Sample type	Sample name	Sample code	Microbiological parameters (CFU/ml = water; CFU/g = Soil)									Remarks	Reference
					TVC	TC	TS	FC	FS	VC	SAC	SHC	PC		
Water															
1.	PRS	Water	OCW	W1	104000	10100	850	1130	210	150	130	180	430		
2.	SOS	Water	OCW	W2	74000	4100	640	870	140	120	120	140	310		
3.	CHB	Water	OCW	W3	18100	1350	200	250	80	80	90	100	200		
4.	CLB	Water	OCW	W4	29100	2060	330	440	110	100	130	140	250		
	Sum				*225200*	*17610*	*2020*	*2690*	*540*	*450*	*470*	*560*	*1190*		
	Average				*56300*	*4402.5*	*505*	*672.5*	*135*	*112.5*	*117.5*	*140*	*297.5*		
	Maximum				*104000*	*10100*	*850*	*1130*	*210*	*150*	*130*	*180*	*430*		
	Minimum				*18100*	*1350*	*200*	*250*	*80*	*80*	*90*	*100*	*200*		
5.	PRS	Soil	OCS	S1	192000	12400	1040	1420	260	180	170	230	920		
6.	SOS	Soil	OCS	S2	106000	6000	910	1100	180	150	150	170	650		
7.	CHB	Soil	OCS	S3	44000	2450	260	330	110	100	100	110	310		
8.	CLB	Soil	OCS	S4	71000	3900	420	780	150	130	140	150	490		
	Sum				*413000*	*24750*	*2630*	*3630*	*700*	*560*	*560*	*660*	*2370*		
	Average				*103250*	*6187.5*	*657.5*	*907.5*	*175*	*140*	*140*	*165*	*592.5*		
	Maximum				*192000*	*12400*	*1040*	*1420*	*260*	*180*	*170*	*230*	*920*		
	Minimum				*44000*	*2450*	*260*	*330*	*110*	*100*	*100*	*110*	*310*		

PRS - Ponmalai railway shed; SOS – Senthaneerpuram oil shed; CHBPB – Chatram Bus stand; CLBPB – Central Bus stand;

OCW – Oil Contaminated Water; OCS – Oil Contaminated Soil

TVC – Total viable count; TC – Total coliforms; TS – Total *Streptococci*; FC – Fecal coliforms; FS – Fecal *Streptococci*; VC – *Vibrio* count; SAC – *Salmonella* count; SHC – *Shigella* count; PC – *Pseudomonas* count;

Clark A, Turner T, Dorothy KP, Goutham J, Kalavati C, Rajanna B (2003) Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India. Ecotoxicology and Environmental Safety 56: 390–397. doi: 10.1016/S0147-6513(03)00098-8. Pubmed: 14575679.

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Vignesh S, Dahms HU, Emmanuel KV, Gokul MS, Muthukumar K, Kim BR, James RA (2014) Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India. Environmental Monitoring and Assessment 186(3): 1875–1887. doi: 10.1007/s10661-013-3501-z. Pubmed: 24292984.

Table 4.15.

Descriptive Statistics-Physiochemical Parameter –Water and Soil Sample

Parameters	N	Mean	SD	Sum	Min	Max
pH-W	16	7.78125	0.35444	124.5	7.2	8.4
pH-S	16	7.9125	0.50183	126.6	6.8	8.6
TDS-W	16	390.3438	155.4656	6245.5	140.2	651.4
TDS-S	16	653.9813	422.5164	10463.7	211.9	1956.8
EC-W	16	661.5996	263.501	10585.59	237.6271	1104.068
EC-S	16	1018.593	465.5205	16297.49	359.1525	1871.301
Salinity-W	16	0.125	0.34157	2	0	1
Salinity-S	16	0.625	0.80623	10	0	3
DO-W	16	5.56875	0.98028	89.1	3.4	6.8
DO-S	16	6.1125	0.92439	97.8	4.1	7.6
BOD-W	16	11.3875	17.4858	182.2	5.5	76.9
BOD-S	16	8.025	1.60395	128.4	6.1	13.5
COD-W	16	12.29375	2.9149	196.7	7.4	17.4
COD-S	16	13.9875	3.04037	223.8	8.4	19.8
TA-W	16	96.80625	39.46197	1548.9	35.8	165.4
TA-S	16	134.2688	54.80315	2148.3	51.2	246.8
TH-W	16	84.0125	40.90574	1344.2	24.4	159.9
TH-S	16	110.7375	55.54013	1771.8	24.3	224.8
Ca-W	16	40.85625	20.78368	653.7	14.2	81.5
Ca-S	16	59.94375	34.86199	959.1	16.1	142.5
Mg-W	16	43.15625	21.57437	690.5	8.4	78.5
Mg-S	16	52.04375	20.39202	832.7	19.8	84.6
Na-W	16	32.15625	13.96152	514.5	12.4	66.3
Na-S	16	53.9375	36.3806	863	15.4	140.5
K-W	16	23.86875	10.71539	381.9	9.5	48.9
K-S	16	28.49375	14.38149	455.9	12.6	63.2
HCO_3-W	16	92.3	37.67321	1476.8	30.6	161.4
HCO_3-S	16	123.5	49.72368	1976	43.5	224.6
Cl-W	16	73.55	33.37772	1176.8	23.8	145.2
Cl-S	16	154.5125	109.4442	2472.2	53.4	465.8
SO_4-W	16	56.6125	27.58635	905.8	18.4	115.6
SO_4-S	16	72.75625	32.59454	1164.1	23.4	137.2
N-NO_2-W	16	5.73375	3.86714	91.74	1.5	15.2
N-NO_2-S	16	6.39375	3.52429	102.3	1.8	13.6
O-PO_4-W	16	6.0625	3.26963	97	2	14.4
O-PO_4-S	16	6.09375	2.0949	97.5	2.6	10.1
Oil-Greece-W	16	7.73125	3.39838	123.7	3	15.4
Oil-Greece-S	16	11.875	5.1094	190	4.1	21.6

Table 4.16. The chromium biosorption studies of two bacterial strains with dry biomass

	Test Strain	Metal solution	pH	Time	Metal level in medium*	
Biosorption study	Bacterial strain 1	5 ppm Chromium solution	4 pH	15 m	Metal Concentration	2.76
					Percentage	55.2
				2 h	Metal Concentration	2.21
					Percentage	44.2
			7 pH	15 m	Metal Concentration	3.25
					Percentage	65.0
				2 h	Metal Concentration	2.92
					Percentage	58.4
		10 ppm Chromium solution	4 pH	15 m	Metal Concentration	6.21
					Percentage	62.1
				2 h	Metal Concentration	5.83
					Percentage	58.3
			7 pH	15 m	Metal Concentration	7.16
					Percentage	71.6
				2 h	Metal Concentration	6.38
					Percentage	63.8
	Bacterial strain 2	5 ppm Chromium solution	4 pH	15 m	Metal Concentration	3.08
					Percentage	61.6
				2 h	Metal Concentration	2.72
					Percentage	54.4
			7 pH	15 m	Metal Concentration	3.48
					Percentage	68.0
				2 h	Metal Concentration	3.12
					Percentage	62.4
		10 ppm Chromium solution	4 pH	15 m	Metal Concentration	7.16
					Percentage	71.6
				2 h	Metal Concentration	6.70
					Percentage	67.0
			7 pH	15 m	Metal Concentration	7.74
					Percentage	77.4
				2 h	Metal Concentration	6.92
					Percentage	69.2

Table 4.17

Descriptive Statistics for Heavy Metal Parameter-Water And Soil

Parameters	N	Mean	SD	Sum	Min	Max
Cd-W	16	0.14125	0.07693	2.26	0.06	0.34
Cd-S	16	0.23312	0.153	3.73	0.1	0.65
Cr-S	16	0.08562	0.04016	1.37	0	0.15
Cr-S	16	0.10563	0.05513	1.69	0	0.22
Cu-W	16	0.15875	0.06459	2.54	0.08	0.32
Cu-S	16	0.22938	0.11156	3.67	0.1	0.52
Fe-W	16	0.72375	0.47614	11.58	0.22	1.84
Fe-S	16	1.28313	0.88363	20.53	0.33	3.46
Ni-W	15	0.05867	0.04502	0.88	0	0.12
Ni-S	16	0.0925	0.05651	1.48	0	0.2
Pb-W	16	0.08812	0.04004	1.41	0	0.14
Pb-S	16	0.125	0.04	2	0.08	0.22
Zn-W	16	0.58625	0.31003	9.38	0.2	1.24
Zn-S	16	0.75	0.44744	12	0.21	1.86

Table 4.18. Percentage of isolated copper resistance strains from oil contaminated regions of Tiruchirappalli, Tamil Nadu

Percentage of growth	Oil contaminated regions - Bacterial isolates (*n* = 60) Copper (Cu) metal solution							
	10 mM		50 mM		100 mM		250 mM	
	N	%	N	%	N	%	N	%
0-10 percentage of growth	-	-	-	-	-	-	-	-
11-20 percentage of growth	-	-	-	-	-	-	-	-
21-30 percentage of growth	-	-	-	-	-	-	-	-
31-40 percentage of growth	-	-	-	-	-	-	-	-
41-50 percentage of growth	-	-	-	-	-	-	1	1.5
51-60 percentage of growth	-	-	-	-	-	-	4	6.5
61-70 percentage of growth	-	-	-	-	07	11.5	18	30
71-80 percentage of growth	-	-	10	16.5	27	45.0	37	62
81-90 percentage of growth	05	8.5	33	55.0	26	43.5	-	-
91-100 percentage of growth	55	91.5	17	21.5	-	-	-	-

	Minimal inhibitory concentration (MIC) of copper – Bacterial strains – (*n* = 60)			
	10 mM	50 mM	100 mM	250 mM
Resistant strains	60	54	43	03

N / n – Numbers; mM – Milli Molar; % - Percentage

Table 4.19. Percentage of isolated chromium resistance strains from oil contaminated regions of Tiruchirappalli, Tamil Nadu

Percentage of growth	Oil contaminated regions - Bacterial isolates (*n* = 60) Chromium (Cr) metal solution							
	10 mM		50 mM		100 mM		250 mM	
	N	%	N	%	N	%	N	%
0-10 percentage of growth	-	-	-	-	-	-	-	-
11-20 percentage of growth	-	-	-	-	-	-	-	-
21-30 percentage of growth	-	-	-	-	-	-	-	-
31-40 percentage of growth	-	-	-	-	-	-	-	-
41-50 percentage of growth	-	-	-	-	-	-	06	10
51-60 percentage of growth	-	-	-	-	01	1.5	11	18
61-70 percentage of growth	-	-	-	-	08	13.5	23	38.5
71-80 percentage of growth	-	-	06	10	31	51.5	20	33.5
81-90 percentage of growth	08	13.5	32	53.5	20	33.5	-	-
91-100 percentage of growth	52	86.5	22	36.5	-	-	-	-

	Minimal inhibitory concentration (MIC) of chromium – Bacterial strains – (*n* = 60)			
	10 mM	50 mM	100 mM	250 mM
Resistant strains	60	51	39	01

N / n – Numbers; mM – Milli Molar; % - Percentage

Table 4.20. The copper biosorption studies of two bacterial strains with dry biomass

	Test Strain	Metal solution	pH	Time	Metal level in medium*	
Biosorption study	Bacterial strain 1	5 ppm Copper solution	4 pH	15 m	Metal Concentration	2.12
					Percentage	42.4
				2 h	Metal Concentration	1.88
					Percentage	37.6
			7 pH	15 m	Metal Concentration	3.06
					Percentage	61.2
				2 h	Metal Concentration	2.82
					Percentage	56.4
		10 ppm Copper solution	4 pH	15 m	Metal Concentration	4.84
					Percentage	48.4
				2 h	Metal Concentration	3.95
					Percentage	39.5
			7 pH	15 m	Metal Concentration	6.18
					Percentage	61.8
				2 h	Metal Concentration	5.65
					Percentage	56.5
	Bacterial strain 2	5 ppm Copper solution	4 pH	15 m	Metal Concentration	2.69
					Percentage	53.8
				2 h	Metal Concentration	2.15
					Percentage	43.0
			7 pH	15 m	Metal Concentration	3.58
					Percentage	71.6
				2 h	Metal Concentration	3.12
					Percentage	62.4
		10 ppm Copper solution	4 pH	15 m	Metal Concentration	6.10
					Percentage	61.0
				2 h	Metal Concentration	5.34
					Percentage	53.4
			7 pH	15 m	Metal Concentration	7.05
					Percentage	70.5
				2 h	Metal Concentration	6.26
					Percentage	62.6

PPM – Parts per million; m – Minutes; h – Hours

** - It indirectly indicated the metal absorption by bacterial strains*

Table 4.21 The chromium biosorption studies of two bacterial strains with dry biomass

	Test Strain	Metal solution	pH	Time	Metal level in medium*	
Biosorption study	Bacterial strain 1	5 ppm Chromium solution	4 pH	15 m	Metal Concentration	2.76
					Percentage	55.2
				2 h	Metal Concentration	2.21
					Percentage	44.2
			7 pH	15 m	Metal Concentration	3.25
					Percentage	65.0
				2 h	Metal Concentration	2.92
					Percentage	58.4
		10 ppm Chromium solution	4 pH	15 m	Metal Concentration	6.21
					Percentage	62.1
				2 h	Metal Concentration	5.83
					Percentage	58.3
			7 pH	15 m	Metal Concentration	7.16
					Percentage	71.6
				2 h	Metal Concentration	6.38
					Percentage	63.8
	Bacterial strain 2	5 ppm Chromium solution	4 pH	15 m	Metal Concentration	3.08
					Percentage	61.6
				2 h	Metal Concentration	2.72
					Percentage	54.4
			7 pH	15 m	Metal Concentration	3.48
					Percentage	68.0
				2 h	Metal Concentration	3.12
					Percentage	62.4
		10 ppm Chromium solution	4 pH	15 m	Metal Concentration	7.16
					Percentage	71.6
				2 h	Metal Concentration	6.70
					Percentage	67.0
			7 pH	15 m	Metal Concentration	7.74
					Percentage	77.4
				2 h	Metal Concentration	6.92
					Percentage	69.2

PPM – Parts per million; m – Minutes; h – Hours

** - It indirectly indicated the metal absorption by bacterial strains*

Table 4.22. The bioaccumulation studies of bacterial strains 1 with living biomass

Biosorption Study – Metal adsorbed by organisms							
Bacterial strain 1 (pH – 7.0 ± 0.2; Tº - 36º C ± 1)							
50 ppm Cu				50 ppm Cr			
36 hours		72 hours		36 hours		72 hours	
Metal Conc	Percentage	Metal Conc	Percentage	Metal Conc	Percentage	Metal Conc	Percentage
Metal adsorbed by microbes							
13.8	27.6	18.2	36.4	11.2	22.4	13.6	27.2
Metal obtained in medium							
36.2	72.4	31.8	63.6	38.8	77.6	36.4	72.8

Metal conc – Metal concentration; Tº - Temperature; PPM – Parts per million; Cu – Copper; Cr – Chromium

Table 4.23 The bioaccumulation studies of bacterial strains 2 with living biomass

Biosorption Study – Metal adsorbed by organisms							
Bacterial strain 2 (pH – 7.0 ± 0.2; Tº - 36º C ± 1)							
50 ppm Cu				50 ppm Cr			
36 hours		72 hours		36 hours		72 hours	
Metal Conc	Percentage	Metal Conc	Percentage	Metal Conc	Percentage	Metal Conc	Percentage
Metal adsorbed by microbes							
10.8	21.6	14.4	28.8	8.6	17.2	11.2	22.4
Metal obtained in medium							
39.2	78.4	35.6	71.2	41.4	82.8	38.8	77.6

Metal conc – Metal concentration; Tº - Temperature; PPM – Parts per million; Cu – Copper; Cr – Chromium

% Metal adsorbed = $(C_i - C_f) / C_i$ x 100

Where, C_i and C_f are the initial and equilibrium metal ion concentrations (mg L^{-1}), respectively.

Table 4.24
The bioaccumulation studies of bacterial strains 1 with living biomass – Field trail

Biosorption Study – Metal adsorbed by organisms								
Bacterial strain 1 (pH – 7.0 ± 0.2; Tº - 35º C ± 2; 200 rpm)								
Cu level in raw sewage sample (ppm)	10 ppm Cu				Cr level in raw sewage sample (ppm)	10 ppm Cr			
	36 h		72 h			36 h		72 h	
	Metal conc	%	Metal conc	%		Metal conc	%	Metal conc	%
0.34	Metal adsorbed by microbes				0.15	Metal adsorbed by microbes			
	1.37	13.7	1.85	18.5		0.94	9.4	1.12	11.2
	Metal obtained in medium					Metal obtained in medium			
	8.97	86.7	8.49	82.1		9.21	90.7	9.03	88.9

Metal conc – Metal concentration; % - Percentage; Tº - Temperature; ppm – Parts per million; Cu – Copper; Cr – Chromium

Table 4.25
The bioaccumulation studies of bacterial strains 2 with living biomass – Field trail

Biosorption Study – Metal adsorbed by organisms								
Bacterial strain 2 (pH – 7.0 ± 0.2; Tº - 35º C ± 2; 200 rpm)								
Cu level in raw sewage sample (ppm)	10 ppm Cu				Cr level in raw sewage sample (ppm)	10 ppm Cr			
	36 h		72 h			36 h		72 h	
	Metal conc	%	Metal conc	%		Metal conc	%	Metal conc	%
0.34	Metal adsorbed by microbes				0.15	Metal adsorbed by microbes			
	1.06	10.6	1.22	12.2		0.72	7.2	0.94	9.4
	Metal obtained in medium					Metal obtained in medium			
	9.28	89.7	9.12	88.2		9.43	92.9	9.21	90.7

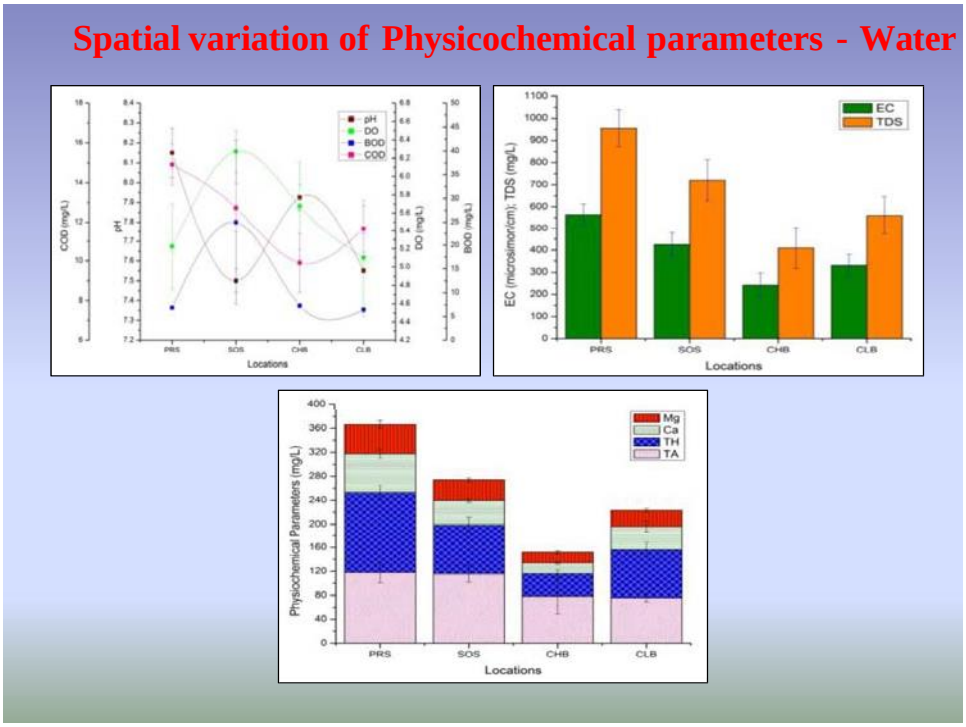
Metal conc – Metal concentration; % - Percentage; Tº - Temperature; ppm – Parts per million; Cu – Copper; Cr – Chromium

% Metal adsorbed = $(C_i - C_f) / C_i$ x 100

Where, C_i and C_f are the initial and equilibrium metal ion concentrations (mg L^{-1}), respectively.

Figure 1

Spatial Overall Physiochemical Parameters -Water



FIGURE;1

Spatial Overall Physiochemical Parameters -Water

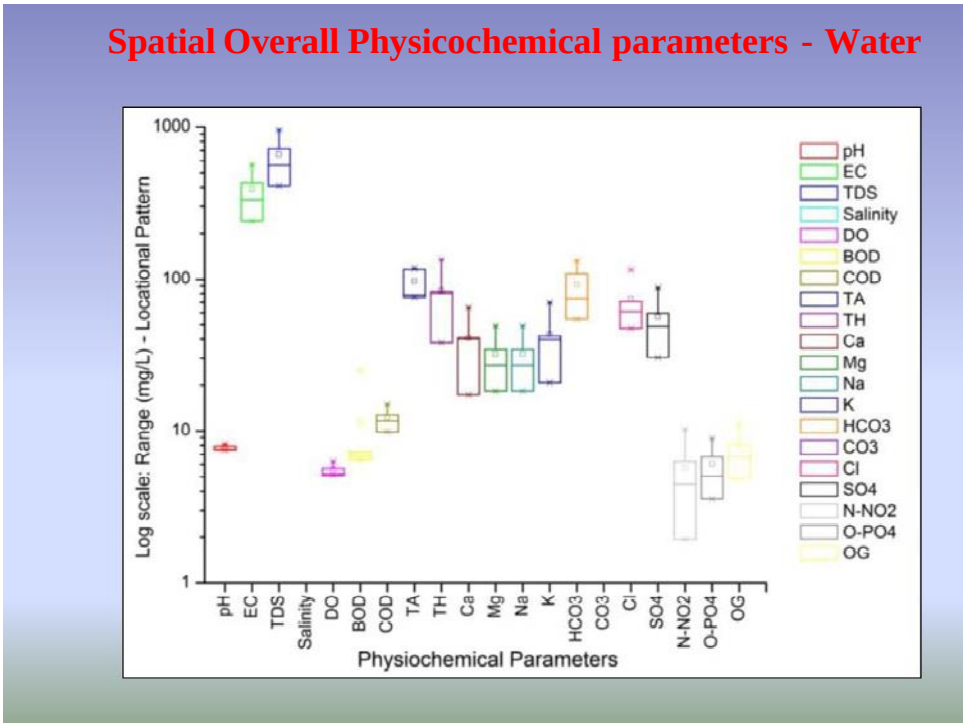


Figure 2

Spatial Overall Physiochemical Parameters - Soil

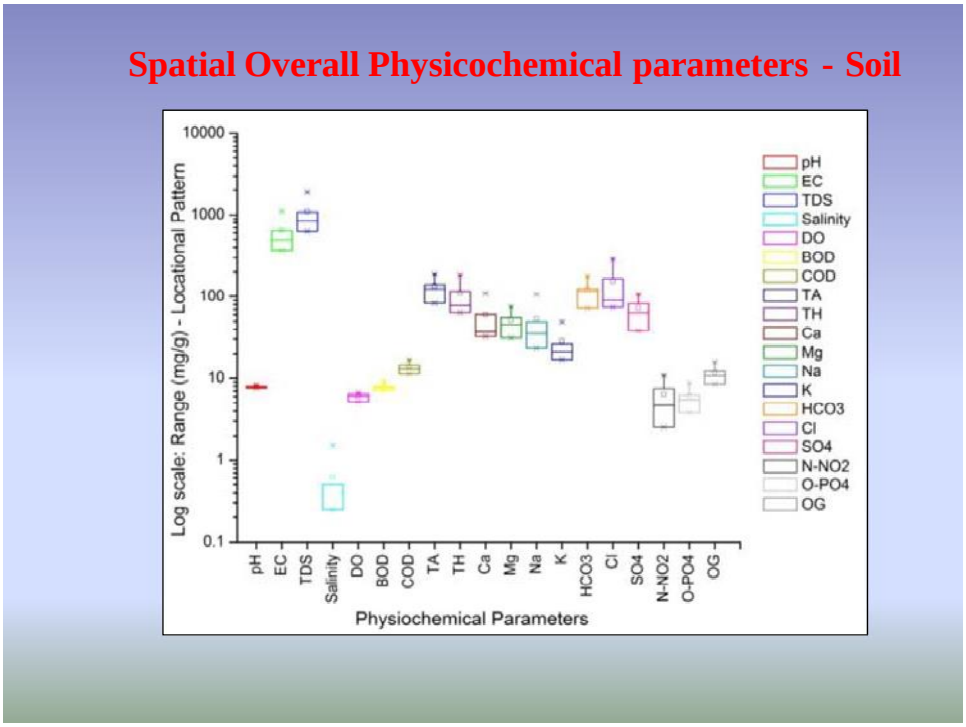
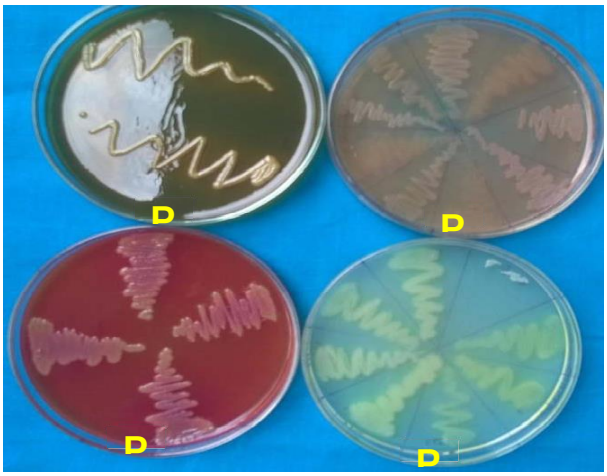


Plate 1 HETEROTROPIC BACTERIAL STUDIES
SELECTIVE MEDIUM USED FOR ENUMERATION OF BACTERIA .



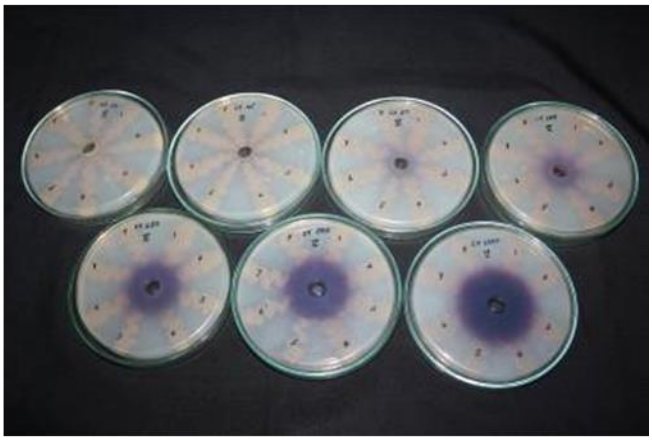
P1 - TCBS-Green colonies
P2 - Macconkey Agar-Pale pink colonies P3 - XLD-Agar-Dark pink colonies
P4 - Cetrimide Agar-Green colonies

Plate 2

HEAVY METALS RESISTANCE BY WELL DIFFUSION METHOD

Radial streaking of isolated bacterial strains in Nutrient Agar medium and performed metal resistance analysis.

1.2.1a. Copper resistant strains in Nutrient Agar medium



2. 2.1b. Chromium resistant strains in Nutrient agar medium

CONCLUSION;

Natural and anthropogenic activities generate large quantities of aqueous effluents containing toxic metals. Many studies have been conducted in recent decades aimed at lowering metal concentrations derived from natural resources. In this study, the microbiological, physico-chemical parameters and heavy metal concentration of water and soil samples showed that this area was highly contaminated by anthropogenic activities espcialy oil contaminated sewage wastes. In general, the high levels of metal and metal resistance in bacteria reflect the widespread use of these metals in these study sites. In addition, considerable effort has been made to develop efficient and cost- effective technologies and apply them to sewage / industrial wastewater treatment. The potential for microorganisms to remove metals from solutions through passive and active mechanisms has been shown to be an interesting approach to metal uptake in polluted waters, and the efficiency of such processes is dependent on the experimental conditions, the target pollutant and various other factors.

The application of this type of bioremediation process in large scale remains, however, a challenge, and a preventive approach to metal pollution problems is therefore encouraged. Further investigations aimed at the identification of the mechanisms involved the characterization of biosorbents, and advances in genetic engineering are required. The copper removal potential of bacterial strain 1 was higher than bacterial strain 2 and also the same pattern follow in the chromium removal methods. Interestingly, the copper was highly

removed by microbes than chromium. The bacterial strain 1 effectively removed the metals from a both field trail and natural (medium + metal solution) samples due to its higher metal tolerance, residual growth rate and efficient metal removal. However, several phases of metal–bacteria interactions remain unexplored and further improvement and application are necessary. The present results indicate that both *Pseudomonas* biomasses may be a suitable material for the removal of copper and chromium ions from the solution. However, desorption experiments remain to be performed to assess the reusability of this low-cost

iosorbent. Furthermore, in view of the practical application of this waste biomass to the treatment of metal bearing matters, this preliminary study needs obviously to be completed by additional experiments concerning in particular the influence of ionic strength and diverse constituents that are frequently found in actual industrial effluents, such as surfactants, complexing agents and other metal ions. These extensions are under investigation and will be reported later.

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