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Impact of heavy metal contamination in different soil towards microbial characteristics and nutrient availability

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ABSTRACT

An intensive investigation for detecting source of the heavy metals contamination in different areas (within industry, at roadside, adjacent agriculture lands) of various industries located at two sites A & B were made. Co-relationships were determined for specific metal contamination in leacheates at sites with total contents of heavy metal at source. At site A, it was observed that the leached fractions were highly correlated with the total contents of all tested heavy metals showing same source of contamination, whereas at site B relation was obtained only in case of Mn i.e 0.99, Zn i.e 0.82 and 0.70 for Cu, while, rest had sparse relationship, showing that the variability in heavy metal contents was caused by the different sources of contamination. The soil samples showed different microbial groups (cfu), in the order Bacteria > Fungi > Actinomycetes > Rhizobium and Azotobacters. The results indicated that the low availability of nutrients (NPK) in soil could be due to the heavy metal contamination which reduced the beneficial microbes such as Rhizobia and Azotobacters in contaminated soil.

KEYWORDS

Heavy metal;
Microbial characteristics;
Nutrient availability;
Soil contamination;
Physico-chemical characteristics.

INTRODUCTION

Soil contamination, with hazardous and toxic chemicals is serious problems, which have been faced by whole world. Maintenance of good soil quality is of prime importance as it may threaten human health through its effect on air quality as well as cause environmental pollution. Consensus about soil quality standards is not yet well established; mainly no single or combined biological and physico-chemical variable is available to reflect the many interacting processes responsible for soil quality[1]. Physical and chemical properties have been extensively used to measure soil quality[2]. However these properties usually change on a time scale (decade) which is too long for measurement purpose.

Accordingly for environmental impact assessment it is pertinent to study the characteristics of soil, the resultant behaviour of the surrounding soil environment. Heavy metals and organic compounds are among the "most important" pollutants present in soil, water, sewage sludge[3,4] etc. Although the total soil concentration of metal is commonly used in soil environmental quality standard[5], but it is useful to predict other parameters too, as the speciation and bioavailability of the metals in soil vary greatly due to soil physico-chemical properties. The most important soil variable affecting the bioavailability of heavy metal is soil pH. Higher pH reduces the bioavailability and toxicity of cadmium and lead[6-9]. Thus soil quality criteria for the trace metals should be based on the bioavailable pool of the elements to ensure adequate environmental protection[10].

During the last few decades extensive attention has been paid to the hazards arising contamination of the environment by heavy metal. Heavy metals are well known to be toxic to most organisms when present in high concentration in the environment. They are known to affect the growth, morphology and metabolism of micro-organisms in soil, even limiting the species composition and microbial reproduction as they cause destruction of the integrity of cell membranes[11-13]. Soil may become contaminated with metals from variety of anthropogenic sources specially mining, smelting of various metal power station, waste discharge, fertilizers, coal burning, heavy metal containing pesticides[14], etc. are the major contributors for pollution. Heavy metal contamination in soil mainly results from the discharge of metal containing waste and waste water from ore refining, production of steel and alloys, metal plating, tannery, wood preservation, pigmentation, glass manufacturer, semiconductor material, feed additives herbicides, insecticides, hematosis additives and veterinary chemicals[15] such as hexavalent chromium is widely used in many industrial process electroplating, wood preservatives etc.[16]. Niragu and Pacyna[17] reported 52,000-112,000 tons of arsenic were released, lead/zinc smelter, increased up to 49 mg/kg[18] annually to soil from anthropogenic sources.

Once incorporated into the soil, they remain for very long period of time, up to of several thousand years[19]. Soil deterioration by metal contamination due to past and present human industrial activity may result in the high exposure. Accumulated toxic heavy metal in surface soils can be transported to different environmental components which may affect directly or indirectly[20] through its enriched trace metal contents in air born particles originating from deep soil, water, plant and dust particles.

The objective of the study was confined to two contaminated sites A & B where road side soil and agriculture soil of the respective area were monitored. Industrial soil sample was compared with road side soil sample as well as agriculture side soil sample. The study investigated the influence of heavy metals (copper, lead, chromium, cobalt, iron, zinc, cadmium, nickel and manganese) on physical, chemical and microbiological characteristics of soil sand their correlation. The linear correlation was also drawn between total heavy metal versus leached heavy metal, and microbes involved for the availability of nitrogen (N), phosphorus (P) and potassium (K) in soil.

MATERIALS AND METHOD

All the chemicals used in the experiment are of analytical grade purchased from Merck, India and Himedia chemicals, Mumbai.

Study site

The study was conducted at two industrial sites A & B at Nagpur, India. Agriculture sample along with road sample were also collected from the respective study areas. Soil sampling was done with the help of post hole soil auger of height 30cm and can dig up to 15cm - 17cm.

Soil sampling strategy

Nine replicate square plots (25m x 25m) were selected for each location. These plots were considered to be true replicates as the distance between them exceeded in the spatial dependence (13 – 15m) of most soil chemical and microbial variables[21]. Five replicate soil samples, 2kg each, were taken from the depth of about 15cm of each plot.

A set of nine bulk samples with controls were collected, four from site A and five from site B, 0-15 cm depth within each of the plots and were kept in the ice box and transported to the laboratory for further analysis. A part of sample was refrigerated at 4^{o}C to avail enumeration of micro flora which was carried out within one month, and rest of the sample was dried and sieved (2 mm mesh) for the determination of various physical and chemical parameters.

Physical analysis

Particle size analysis

The international pipette method[22] was adopted for the determination of particle size analysis. In short, soils were first treated with hydrogen per oxide (30%) to remove organic matter. Particle size distribution was determined (i) by successive sieving to determine the proportion of coarse rock fragments (20-100mm), fine rock fragments (gravel: 2-20mm) and sand (0.05-2mm) and (ii) by sedimentation (Robinson pipette method) of the <0.05mm fraction for determining proportions of fine silt (2 - 20μm) and clay (<2μm).

Chemical analysis

Soil pH, electrical conductivity and soluble ions

Soil pH and electrical conductivity (EC) were determined by standard method[22].

Cation exchange capacity

Cation exchange capacity (CEC) is estimated by leaching the soil with 1N ammonium acetate 7.0 pH, the leachate were use for the estimation of exchangeable cations, i.e. calcium, magnesium, sodium and potassium. Calcium and magnesium were estimated by Versenate method, while sodium and potassium were estimated by flame photometric method[22]. Soil was thereafter washed with isopropyl alcohol for chloride removal followed by leaching with 10% potassium chloride pH 2.5. Potassium chloride leachate were use for CEC determination by distillation through Kjeldhal distillation assembly, followed by titration against 0.02N sulphuric acid[23].

Nutrient status of soil

Total nitrogen and available nitrogen was determined by Kjeldhal's method, total phosphorus and available phosphorus by Olsen's method[24] whereas total potassium and available potassium by flame photometric method[23]. And organic carbon and organic matter were estimated by Walkey and Black method[24].

Toxicity of soil

Toxicity of soil was estimated by determining the concentration of heavy metals present in soil. Total heavy metals were determined by digesting soil in acid mixture (1:2 perchloric acid and nitric acid) followed by filtration, and analyzed on atomic adsorption spectrophotometer (AAS). While toxicity characteristic leaching procedure (TCLP) was use for the determination of leached heavy metals. In short, soil samples were shaked with water, pH was measured. Then 1N hydrochloric acid was added, covered and boil. Sample was cooled and type I solution (64.3ml 1N sodium hydroxide and 5.7ml glacial acetic acid to 1000ml distilled water pH 4.93) was added. The sample was agitated for 18hrs followed by centrifugation at 8000 rpm and filtration, then analyzed by atomic adsorption spectrophotometer (AAS).

Microbiological analysis

Enumeration of soil micro flora was determined by using pour plate method. The experiment was conducted in triplicates. Total viable count in soil were estimated using "nutrient agar medium" (5g peptone, 3g beef extract and 3g sodium chloride 20g bacteriological agar in 1000ml distilled water pH 7.0[25]). Soil fungi were determined using "Martin's Rose Bengal agar medium" (10g dextrose, 5g peptone, 1g potassium dihydrogen phosphate, 0.5g magnesium sulphate, 33.3mg Rose Bengal, 0.3g antibiotic streptomycin and 20g bacteriological agar in 1000ml distilled water pH 5.5, Martin 1950). Soil actinomycetes were estimated using "starch casein agar medium" (10g starch, 0.3g casein, 2g potassium nitrate, 2g sodium chloride, 2g dipotassium hydrogen phosphate,0.05g magnesium sulphate penta hydrate and 20g bacteriological agar in 1000ml distilled water pH 5.5, Jensen 1930). Soil Azotobacter population were grown on "Jenson's medium" (20g sucrose, 1g di-potassium hydrogen phosphate, 0.05g magnesium sulphate penta hydrate, 0.5g sodium chloride, 0.01g ferrous sulphate, 0.005g di-sodium magnesium oxide, 2g calcium carbonate and 20g bacteriological agar in 1000ml distilled water pH 7.0, Jenson 1942). And soil rhizobium were estimated by using "yeast extract mannitol agar medium" (10g mannitol, 0.5g di-potassium hydrogen phosphate, 0.2g magnesium sulphate penta hydrate, 0.1g sodium chloride, 1g yeast extract and 20g bacteriological agar in 1000ml distilled water pH 7.0[26]).

Statistical analyses were carried out with XLfit 5 software. Linear correlations were used to test the relationships between variables. The confidence limits of the results are given at the $p < 0.05$.

RESULT AND DISCUSSION

Industrial soil samples from two sparsely located sites viz; A & B were assessed and compared with agriculture and road side soil surrounding the same sites.

Physical characteristics

Air-dried and sieved samples have been used for determination of physical characteristics of soil such as particle size distribution of study zone, in terms of percentage of sand, silt and clay. The textural diagram was generated using "SEE Soil Class 2.0 version based on United States Department of Agriculture (USDA) classification of soils (Figure 1). The soil samples had almost similar properties concerning texture, except sample 1 and 2 which had more sand content while no variation was observed in pH and EC (TABLE 1).

Chemical characteristics

Chemical characteristics of soil is given in TABLE 1. The classification of soil and their relationship with adsorptivity and productivity based on cation exchange capacity (CEC) is presented in TABLES 1a and 1b. According to CEC, the productivity and adsorptivity of soil sample 1 and 2 was found to be low because soil having more sand content and was not able to hold the nutrients as they are generally associated with the finest fractions of the soils[27]. Apart from the sample 1 and 2 all soil samples have moderate to high productivity and adsorptivity level. Total and leached fractions were presented in TABLES 2 and 3 respectively, in which Cd seems to be more pronounced crossing the limit causing pollution.

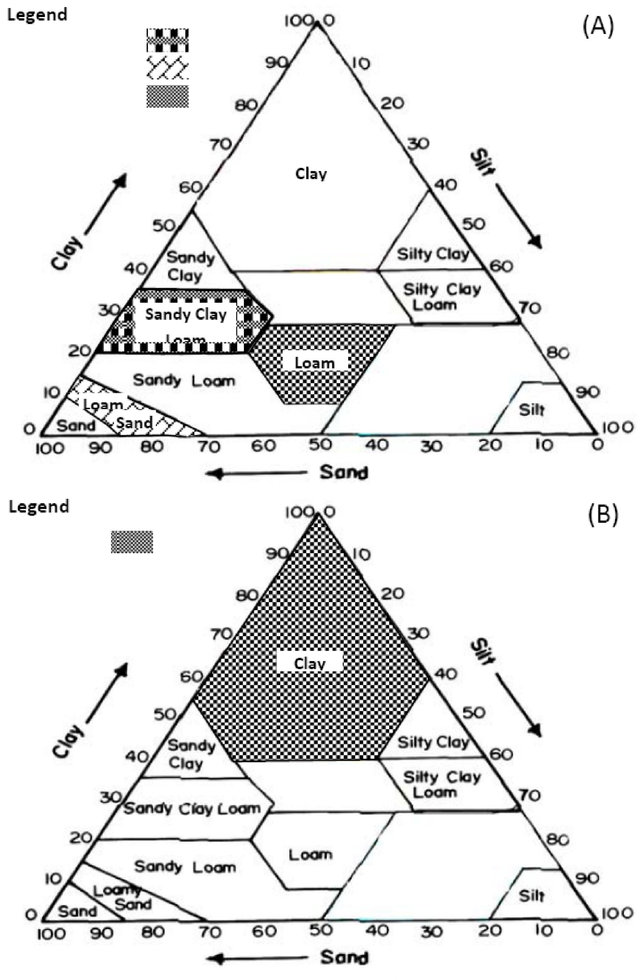

Figure 1 : Texture diagram of site A and site B

Soil microbiology

Bacteria are the biological indicators; they are valuable to assess the soil quality. Brookes and McGrath (1984) have provided evidence that heavy metals decrease the proportion of microbes. Heavy metals generally exert an inhibitory action on soil micro-organisms by displacing essential metal ions, blocking essential functional groups, or by modifying the active conformation of biological molecules[29,30].

From TABLE 1, fungi appear to be more tolerant to heavy metals than Bacteria and Actinomycetes[31,32]. Asymbiototic nitrogen fixers seem to be more sensitive to small amount of heavy metals[33-35]. Our results also support the idea that this group of soil bacteria plays an important role in monitoring the possible impact of heavy metal contamination. Our study demonstrated that changes in soil conditions due to heavy metal contamination have large negative results of the microbial counting. Consequently, the number of soil microbes would be reduced. Our results strongly suggest that soil micro-organisms varied with the contamination gradient.

In this investigation, the total number of cfu of total Bacteria, Fungi, Actinomycetes, asymbiotic Nitrogen fixers and symbiotic nitrogen fixers was reduced in the contaminated site. Thus, nitrogen fixing bacteria seems to be more sensitive to heavy metal contamination than the other microbial group under evaluation, undergoing a decrease in population size of about 60% - 75%. Difference in the viable counts of Fungi and Actinomycetes were also significant; however, these two microbial groups seem to be less sensitive to the presences of heavy metals. The heavy metal tolerance ca-

TABLE 1 : Physico-chemical and microbiological characteristics of soil (mean ± standard deviation, cfu^{-g}, $n = 3$)

Sampling Plot	pH (1:2)	EC (dS/m)	Organic Matter(%)	CEC Cmol (p^+) kg^{-1}	TVC (10^5 cfu/g)	Fungi (10^4 cfu/g)	Actinomycetes (10^4 cfu/g)	Rhizobium (10^3 cfu/g)	Azotobacter (10^3 cfu/g)
1	5.72 ± 0.22	1.47 ± 0.04	1.29 ± 0.07	07.30 ± 0.22	012.00 ± 02.00	012.33 ± 01.53	03.00 ± 0.00	001.67 ± 1.16	001.67 ± 1.53
2	6.23 ± 0.12	0.92 ± 0.08	1.27 ± 0.04	15.90 ± 0.10	040.00 ± 10.00	015.33 ± 02.52	03.33 ± 0.58	002.67 ± 1.15	001.33 ± 1.53
3	6.39 ± 0.15	6.71 ± 0.07	1.87 ± 0.04	36.29 ± 0.09	252.67 ± 11.02	010.33 ± 02.52	15.67 ± 3.51	007.67 ± 4.16	003.00 ± 4.58
4	6.26 ± 0.07	1.58 ± 0.02	0.97 ± 0.05	29.12 ± 0.29	083.33 ± 10.26	017.67 ± 02.52	19.00 ± 4.00	005.33 ± 7.02	005.33 ± 4.73
Road side	6.15 ± 0.04	5.94 ± 0.03	1.26 ± 0.04	44.81 ± 0.19	330.00 ± 36.06	034.67 ± 06.03	05.67 ± 3.06	001.67 ± 3.21	003.33 ± 1.53
Agriculture	5.86 ± 0.03	5.95 ± 0.03	1.55 ± 0.33	30.92 ± 0.08	990.00 ± 45.83	110.00 ± 26.46	08.00 ± 4.58	160.67 ± 4.51	120.33 ± 2.52
5	6.44 ± 0.04	6.77 ± 0.02	3.86 ± 0.13	21.55 ± 0.25	163.33 ± 15.28	15.67 ± 5.13	05.00 ± 3.00	001.33 ± 0.58	006.33 ± 2.08
6	6.43 ± 0.03	0.47 ± 0.04	0.96 ± 0.05	26.48 ± 0.24	476.67 ± 25.17	30.00 ± 10.00	18.67 ± 2.08	005.00 ± 4.00	005.67 ± 1.53
7	6.47 ± 0.03	0.25 ± 0.05	0.95 ± 0.05	51.34 ± 0.14	176.67 ± 25.17	35.00 ± 15.00	15.67 ± 4.04	004.33 ± 5.58	002.33 ± 4.51
8	6.59 ± 0.02	0.64 ± 0.10	0.95 ± 0.05	38.97 ± 0.44	336.67 ± 15.28	45.67 ± 16.01	14.00 ± 2.00	005.33 ± 1.53	002.33 ± 3.06
9	6.22 ± 0.02	0.60 ± 0.07	2.19 ± 0.06	42.86 ± 0.23	123.33 ± 25.17	33.00 ± 14.53	12.33 ± 2.52	007.67 ± 2.52	002.00 ± 4.00
Road side	5.94 ± 0.03	3.10 ± 0.10	1.27 ± 0.05	45.89 ± 0.17	240.00 ± 36.06	38.33 ± 11.37	04.67 ± 3.06	004.33 ± 3.21	004.33 ± 3.06
Agriculture	5.95 ± 0.03	0.89 ± 0.02	1.57 ± 0.35	35.50 ± 0.39	863.33 ± 70.95	170.00 ± 45.83	15.00 ± 6.56	190.00 ± 5.29	150.67 ± 5.86

cmol (p^+) kg^{-1} is centimol (proton ions) per kilogram, TVC is total viable count and cfu is colony forming unit

pacity was found in the order Fungi > Actinomycetes > asymbiotic Nitrogen fixers and symbiotic Nitrogen fixers, total bacterial count also seems to be pronounced.

Nutrient status of soil

A presence of organic matter, organic carbon, nitrogen, phosphorus and potassium shows the nutrient status of soil and its importance from fertility point of view. Organic matter present in the soil (TABLE 1) influences its physical and chemical properties. Organic matter commonly accounts as one third or more of the cation exchange capacity of surface soil. It is also responsible for the stability of soil aggregates.

The availability of nutrient content was very low as compared to the agricultural soil availability, depicted in Figure 2. At site A, the decrease in availability was about 40.5% - 20.23% in nitrogen, 25% - 4.3% in phosphorus and 25.6% - 20.5% in potassium while road side soil sample showed the decrease of about 28.4%, 22.3% and 25.7% respectively. Same at site B, i.e. 33% - 13.3%, 27.36% - 17.38% and 34.22% - 20.17% decrease whereas, road side soil sample showed 41%, 18% and 44% decrease in nitrogen, phosphorus and potassium respectively. The availability of nutrients found to be less than the road side sample too, the decrease in availability may be attributed due to the inactivation or reduction in number of micro-organism due to heavy metals contamination, necessary for the nutrient transformation i.e. organic forms are transferred to their respective inorganic forms and thus,

TABLE 1a : Relationship of CEC with productivity

CEC	Range (cmol (p^+) kg^{-1})	Productivity	Soil samples
Very low	< 10	Very low	1
Low	10 – 20	Low	2
Moderate	20 – 50	Moderate	3, 4, 5, 6, 7, 9
High	> 50	High	8

cmol (p^+) kg^{-1} is centimol (proton ions) per kilogram

TABLE 1b : Relationship of CEC with adsorptivity

CEC	Range (cmol (p^+) kg^{-1})	Adsorptivity	Soil samples
Limited	<10	Limited	1
Low	10-20	Moderate	2
Moderate	20-30	High	4, 5, 6
High	> 30	Very high	3, 7, 8, 9

cmol (p^+) kg^{-1} is centimol (proton ions) per kilogram

TABLE 2

Sampling Plot	Cd	Cr	Co	Cu	Fe	Mn	Ni	Pb	Zn
1	4.47 ± 0.35	70.80 ± 0.26	36.87 ± 0.16	391.19 ± 11.41	3731.77 ± 0.33	1992.55 ± 0.56	60.67 ± 0.17	57.31 ± 0.34	70.76 ± 0.26
2	3.90 ± 0.20	78.80 ± 0.50	53.67 ± 0.28	230.93 ± 00.12	3613.68 ± 0.30	1915.50 ± 0.44	865.30 ± 0.33	54.86 ± 0.23	524.89 ± 0.38
3	2.17 ± 0.15	53.90 ± 0.17	19.01 ± 0.11	063.94 ± 00.31	3239.66 ± 0.32	594.71 ± 0.33	44.55 ± 0.42	79.30 ± 0.24	102.70 ± 0.25
4	2.83 ± 0.15	90.64 ± 0.39	63.47 ± 0.44	147.27 ± 00.29	3545.66 ± 0.26	804.43 ± 0.56	74.52 ± 0.41	24.66 ± 0.72	169.90 ± 0.25
Road side	4.00 ± 0.20	76.55 ± 0.34	38.88 ± 0.18	89.61 ± 00.35	3436.80 ± 0.35	1071.30 ± 0.33	87.54 ± 0.33	10.87 ± 0.35	82.20 ± 0.27
Agriculture	0.79 ± 0.02	14.64 ± 0.29	57.61 ± 0.53	106.65 ± 00.29	3175.24 ± 0.36	13.53 ± 0.42	13.46 ± 0.22	1.21 ± 0.14	112.35 ± 0.32
5	3.77 ± 0.25	22.76 ± 0.25	30.49 ± 0.31	152.92 ± 00.31	3413.94 ± 0.58	691.15 ± 0.64	64.8 ± 0.22	17.80 ± 0.26	162.15 ± 0.26
6	4.60 ± 0.30	36.93 ± 0.25	47.66 ± 0.26	151.79 ± 00.25	3471.89 ± 0.50	1130.54 ± 0.53	91.66 ± 0.31	11.62 ± 0.35	134.82 ± 0.38
7	4.70 ± 0.30	97.31 ± 0.34	61.92 ± 0.11	124.89 ± 00.27	3490.77 ± 0.34	1540.50 ± 0.48	124.68 ± 0.28	21.64 ± 0.49	103.16 ± 0.29
8	4.07 ± 0.20	60.74 ± 0.28	36.82 ± 0.28	107.92 ± 00.21	3448.79 ± 0.36	1024.22 ± 0.28	79.26 ± 0.28	12.98 ± 0.27	100.61 ± 0.46
9	3.87 ± 0.25	73.43 ± 0.48	38.67 ± 0.28	115.55 ± 00.44	3463.79 ± 0.35	984.27 ± 0.28	87.45 ± 0.19	11.78 ± 0.35	126.44 ± 0.41
Road side	2.53 ± 0.40	21.41 ± 0.44	20.65 ± 0.41	108.68 ± 00.28	3319.76 ± 0.34	588.32 ± 0.27	50.82 ± 0.28	10.60 ± 0.33	96.51 ± 0.32
Agriculture	0.96 ± 0.04	11.59 ± 0.37	76.26 ± 0.29	281.88 ± 00.24	3199.12 ± 0.19	4.43 ± 0.17	11.42 ± 0.21	-	210.31 ± 0.45

Total heavy metals in soil samples (mean ± standard deviation, mg kg^{-1} n = 3)

they help in fixation and enrichment of soil.

Metal content and their correlation

Soil sample were analyzed for total metal content and its leached fractions such as cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) presented in TABLE 2 and 3 respectively and their correlationship were depicted in Figure 3 & 4.

The correlation between total and leached metal contents at site A were highly significant with respective values of 0.94 for Zn, 0.90 for Cd and Cu and 0.86 for Ni and perfect relation with Mn, whereas Pb had significant relation with respective value of 0.75 and Cr, Co and Fe were sparsely significant with 0.21, 0.41 and 0.31 values respectively presented in Figure 4. While

TABLE 3 : Leached heavy metals in soil samples (mean ± standard deviation, mg kg^{-1} n = 3)

Sampling Plot	Cd	Cr	Co	Cu	Fe	Mn	Ni	Pb	Zn
1	0.62 ± 0.06	6.45 ± 0.38	5.05 ± 0.45	98.79 ± 0.56	355.95 ± 0.89	265.22 ± 00.80	4.05 ± 0.55	3.27 ± 0.53	1.14 ± 0.05
2	0.32 ± 0.08	28.93 ± 0.46	3.91 ± 0.41	39.00 ± 0.21	580.86 ± 0.53	255.16 ± 00.63	10.12 ± 0.36	5.03 ± 0.16	96.04 ± 0.51
3	0.11 ± 0.04	5.74 ± 0.28	2.59 ± 0.41	33.03 ± 0.50	314.65 ± 0.50	34.23 ± 25.60	7.27 ± 0.40	3.86 ± 0.35	10.93 ± 0.32
4	0.81 ± 0.05	5.17 ± 0.35	3.87 ± 0.25	29.18 ± 0.38	689.73 ± 0.43	25.20 ± 00.69	5.19 ± 0.42	0.63 ± 0.15	52.07 ± 0.24
Road side	0.57 ± 0.06	3.80 ± 0.24	1.66 ± 0.40	4.94 ± 0.33	809.94 ± 0.67	100.94 ± 00.75	4.98 ± 0.39	0.12 ± 0.05	4.67 ± 0.46
Agriculture	0.20 ± 0.06	2.80 ± 0.30	4.95 ± 0.34	10.13 ± 0.37	198.15 ± 0.37	2.02 ± 00.43	2.83 ± 0.40	0.20 ± 0.04	15.73 ± 0.42
5	0.4 ± 0.05	3.11 ± 0.73	2.16 ± 0.34	57.93 ± 0.34	647.67 ± 0.42	25.87 ± 00.33	9.12 ± 0.38	0.22 ± 0.08	54.88 ± 0.55
6	0.58 ± 0.03	9.03 ± 0.16	3.14 ± 0.32	19.17 ± 0.32	259.84 ± 0.26	110.20 ± 00.40	5.27 ± 0.38	0.61 ± 0.06	14.16 ± 0.66
7	0.28 ± 0.07	5.05 ± 0.17	2.05 ± 0.49	20.03 ± 0.55	490.19 ± 0.74	209.09 ± 00.74	12.88 ± 0.43	0.52 ± 0.06	18.91 ± 0.38
8	0.58 ± 0.04	4.89 ± 0.31	6.48 ± 0.45	10.88 ± 0.35	429.92 ± 0.38	985.10 ± 00.54	5.13 ± 0.53	1.68 ± 0.11	11.90 ± 0.40
9	0.27 ± 0.05	27.56 ± 0.45	2.89 ± 0.32	15.98 ± 0.49	701.05 ± 0.49	101.82 ± 00.50	1.11 ± 0.06	0.17 ± 0.05	24.88 ± 0.58
Road side	0.29 ± 0.05	1.66 ± 0.40	0.11 ± 0.03	10.79 ± 0.30	459.92 ± 0.37	56.13 ± 00.33	4.55 ± 0.46	0.3 ± 0.05	96.67 ±0.45
Agriculture	0.11 ± 0.04	1.16 ± 0.29	2.96 ± 0.49	5.18 ± 0.26	215.18 ± 0.40	0.91 ± 00.03	1.89 ± 0.40	-	45.83 ± 0.24

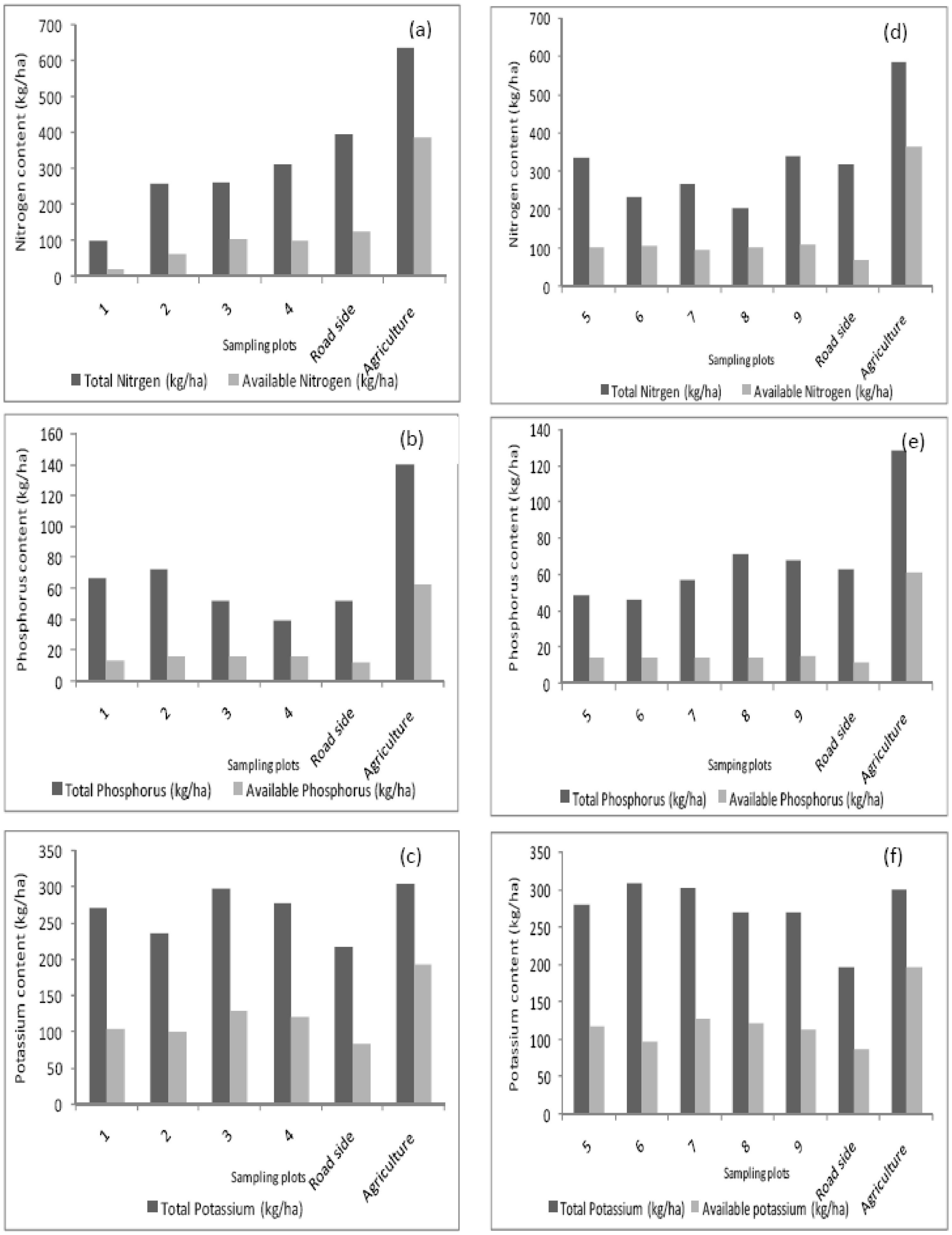

Figure 2 : Total and available nutrient contents, (a), (b) and (c) of site A and (d), (e) and (f) of site B soil samples

in site B, perfect correlation was found only for Mn i.e. 0.99, Zn and Cu showed significant relation with respective values of 0.83 and 0.70 respectively, and sparsely significant relation of 0.12 for Cd, 0.27 for Cr, 0.32 for Co, 0.41 for Fe, 0.46 for Ni and 0.23 for Pb presented in Figure 4 ($P < 0.05$ in all case), showing that the variability in heavy metal contents was caused by the different sources of contamination.

Total metal content and their correlation with the characteristics of the soil

Positive correlation was found between total heavy metal content and soil characteristics. At site A, high correlation was found between the pH and EC with respective value of 0.96 for Cu and 0.93 for Fe. Highly significant relation were found in exchangeable Ca^{++} with the respective value of 0.97 for Cr and Pb and 0.92 for Co; whereas exchangeable Mg^{++} were sparsely significant in all respect except Pb of 0.88 relation. In case of Na^{+} highly significant correlation was found only for Cu of 0.90 and with K^{+} perfect correlation for Cr and highly significant relation for Co of 0.98 and Pb of 0.96. CEC has perfect correlation of 1.00 for Cd, highly significant relation of 0.98 and 0.97 for Cu and Mn respectively

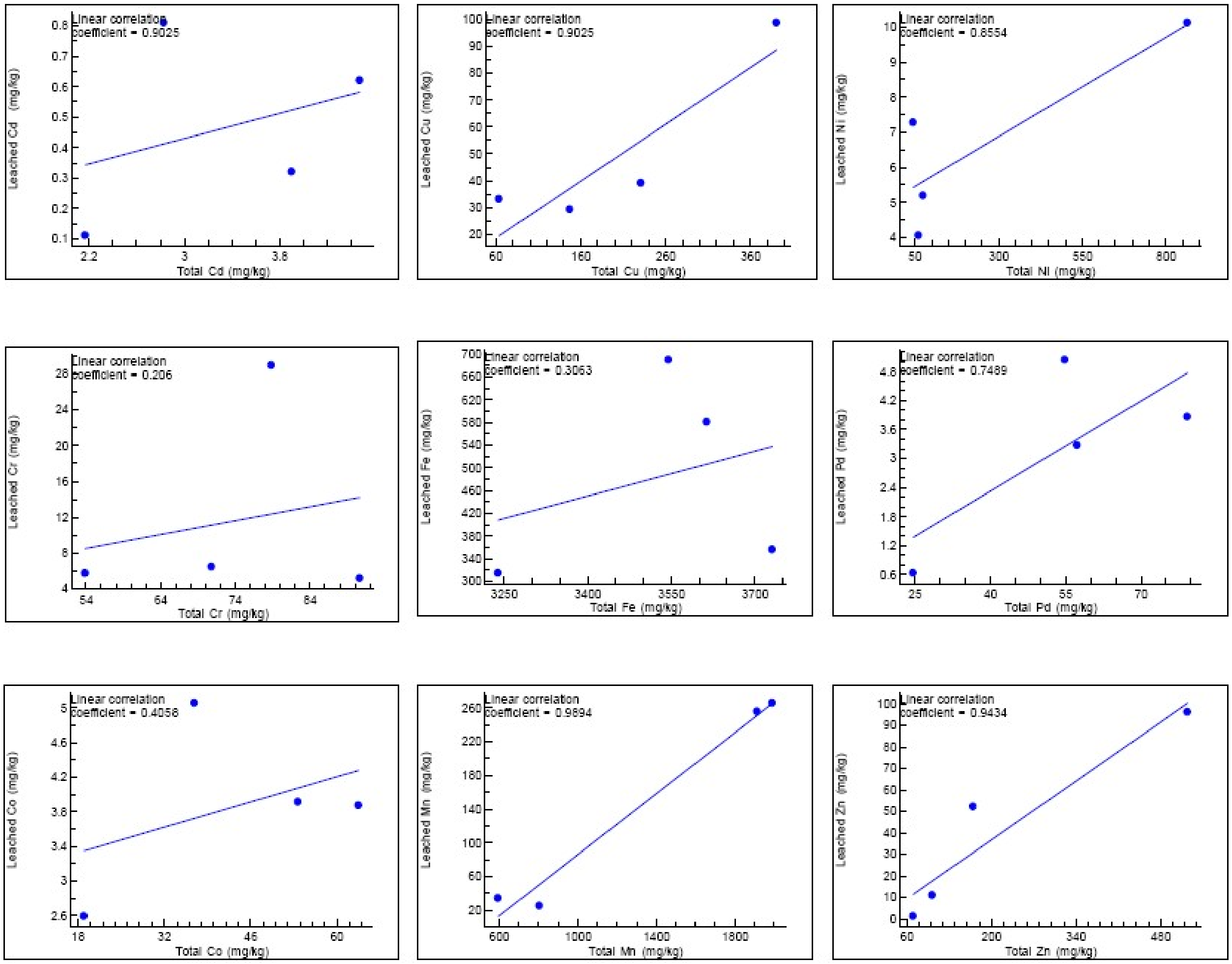

Figure 3 : Relationships between total and leached metal contents in soil of site A Confidence limit is 0.05

and 0.92 for Fe, whereas, exchangeable sodium percentage (ESP) were significantly correlated with all metals.

Organic matter and organic carbon were highly significant with respective values of 0.97, 0.95 and 0.94 for Cr, Pb and Co respectively. Available nitrogen has highly significant correlation of 0.95 for Cr and 0.96 for Co, sparsely significant relation was found with available phosphorus except for Pb of 0.86 significant relation and in available potassium highly significant relation were found with respective value of 0.98 for Cd and Mn and 0.96 for Cu and of 0.85 for Fe. The bacteria were significantly correlated with all the heavy metals except the highly significant correlation of 0.95 for Fe (data not shown).

In site B, sparsely significant relation were found in EC except for Fe of 0.88. While, no relation was observed in pH for Zn and very sparse relation with all other metals. Exchangeable cations too had significant relation except Mg^{++} having perfect relation with Cr and no relation in K^+ for Cd. CEC was significantly related with all metals and perfectly related with Cr. ESP has sparse relation with all metals.

Significant relation was observed in organic matter and organic carbon. Whereas, available nitrogen, phosphorus and potassium content had sparse relationship, and no relation was found between available potassium and Fe. Highly significant relation were seen in Rhizobium sp. with respective value of 0.95 for Fe, 0.91 for Mn, 0.9 for Cr, 0.89 for Ni and 0.85 for Zn. Actinomycetes and Fungi showed significant relationship except Fungi having high significant relation of 0.94 for Zn. Total viable count and Azotobacter had sparsely significant relationship (data not shown).

Leached metal contents and their correlation with the characteristics of the soil

Positive relation was found between leached heavy

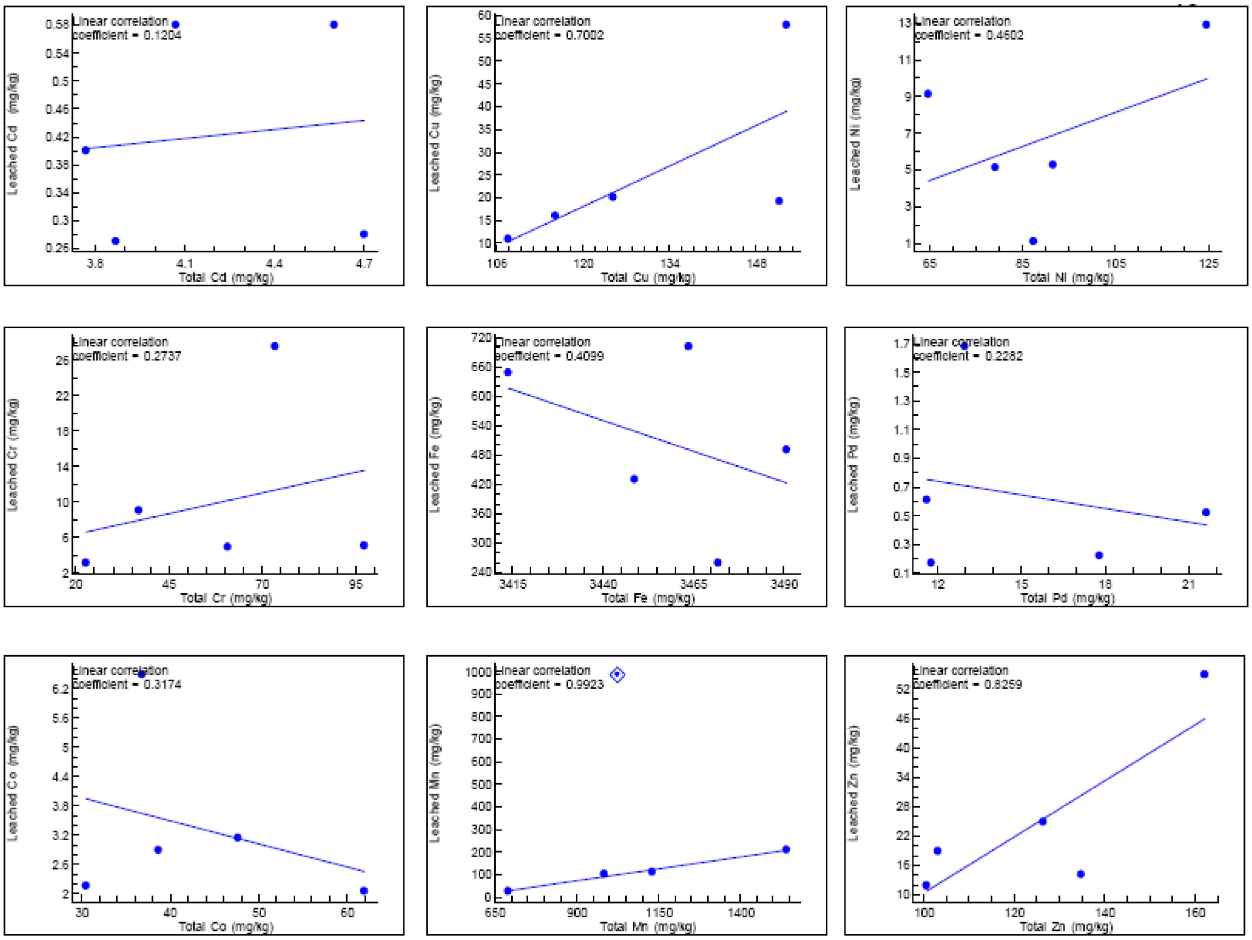

Figure 4 : Relationships between total and leached metal contents in soil of site B Confidence limit is 0.05

metals and soil characteristics (data not shown). At site A, pH had no relation with Pb but highly significant relation of 0.97 for Cu and 0.91 for Co, whereas EC showed sparsely significant relationship. Highly significant relation was found between exchangeable Ca^{++} and Mg^{++} versus Cd of 0.93 and 0.92 respectively, K^{+} versus Fe of 0.91. CEC showed highly significant relation with respective values of 0.94 for Mn, 0.91 for Co. Hence, perfect relationship was obtained between ESP versus Cu, while highly significant relationship was found with respective value of 0.92 for Cd, 0.87 for Ni and 0.86 for Co.

Sparsely significant relation was found between organic matter, organic carbon with all metals. Significant relationship was found between available nitrogen versus all metals, highly significant relation was found between available phosphorus of 0.95 for Pb and highly significant relationship with available potassium of 0.90 for Mn and 0.86 for Co.

Total viable count showed highly significant relation with Cd and Co with respective value of 0.86 and 0.92 respectively. Fungi, Actinomycetes, Rhizobium and Azotobacter had highly significant relation of 0.95 for Co in Fungi, Actinomycetes had 0.88 for Cd and 0.94 for Ni, Rhizobium had 0.93 for Cd and 0.87 Ni and Azotobacter had 0.96 for Ni.

At site B, pH showed highly significant relation of 0.9 for Co and 0.88 for Cr, and EC had 0.97 for Cu and 0.96 for Zn. Exchangeable Ca^{++} had significant relation of 0.87 for Co and 0.86 for Cu and Zn, sparse relationship between Mn^{++} and all metals, highly significant relation between Na^{+} and Co, Mn and Pb with respective value of 0.97, 0.98 and 0.96 respectively, perfect relationship between K^{+} versus Zn. CEC had sparse relation whereas, highly significant relation of 0.98 for Mn, 0.97 for Co and 0.96 for Pb in case of ESP.

Available nitrogen had highly significant relation for Pb and Zn of 0.90 and 0.87 respectively, available phosphorus showed highly significant relation of 0.98 for Cr and sparse relationship between available potassium versus all metals. Organic matter and organic carbon was found to have highly significant relation for Zn of 0.97.

Perfect relation was obtained between total viable count versus Fe and Pb and highly significant relation with Cd and Co of 0.89 and 0.91 respectively. Fungi had highly significant relationship for Cu and Zn of 0.90 and 0.88 respectively. Actinomycetes and Azotobacter had 0.92 relations with Zn and Rhizobium had significant relationship with all metals.

CONCLUSION

The leached fractions were highly correlated with the total contents of heavy metals with respective values of 0.98 for Mn, 0.94 for Zn, 0.90 for Cd and Cu, 0.86 for Ni at Hingna MIDC soils. These correlations showed that the variability in heavy metal contents was caused by the same source of contamination. And at site B soils, perfect relation was obtained only in case of Mn i.e 0.99 and significant relation of 0.82 for Zn and 0.70 for Cu, while, rest have sparse relationship, showing that the variability in heavy metal contents were caused by the different source of contamination. Total metal contents and leached fractions both were positively correlated with physico-chemical and microbial characteristics of soils. Quantitative analysis of soil microbial population showed marked decrease in different microbial groups of contaminated soil samples. The sensitivity of different microbial group were in the order of total viable Bacteria > Fungi > Actinomycetes > Rhizobium and Azotobacters. The availability of nutrients were very low in soil due to the heavy metal contamination, followed by reduction in microbial activity.

From the present study conclusion can be drawn that soil samples of site A were more polluted than site B, although both areas were near to permissible limit. Determining corelationship of the heavy metal contaminations and their sources at any industrial belt help to understand their impact on environmental risks.

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