Potentially toxic metal contamination and enzyme activities in soil around chromite mines at Sukinda Ultramafic Complex, India

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Binaya Kumar Pattnaik, Sk. Md. Equeenuddin *

Department of Earth and Atmospheric Sciences, National Institute of Technology, Rourkela, 769008, Odisha, India

A B S T R A C T

Mining and processing of chromite ore resulting in degradation of soil quality. Sukinda ultramafic complex in Odisha hosts the biggest chromite deposit of India. Therefore, this study carried out to assess the level of various metal concentration, their source and impact of metals on soil enzyme activities. The soil is mostly characterized as acidic to neutral with low organic carbon. Along with the dominance of quartz in soil, common mineral phases identified are clinochlore, lizardite, talc, actinolite, illite, goethite, chromite and maghemite. Average concentrations of Cr, Co, Cu, Mn, Ni, Pb and Zn in soil are 7646 mg/kg, 124 mg/kg, 47 mg/kg, 1791 mg/kg, 780 mg/kg, 18 mg/kg and 77 mg/kg respectively. Concentration of total Cr(VI) in soil varies from 1.45 to 16.7 mg/kg with an average of 7.3 mg/kg. High level of Cr, Ni, Co and Mn in soil is attributed to the weathering of ultramafic rocks. However, Cr(VI) is expected to be derived due to the oxidation of Cr(III) by Mn-oxides as higher concentration of Mn is available in soil. Based on the pollution and contamination index, soil in the area nearby mines is severely contaminated by metals. Enzyme activities are significantly negatively correlated with all metals except Pb. The order of the decrease in the activities is as follows: urease > acid phosphatase ≥ dehydrogenase > β-glucosidase ≥ alkaline phosphatase.

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1. Introduction

Mining of chromite ore and its processing produces huge quantity of waste materials such as overburden, tailing and slags, and thereby releases metals into the nearby environment and leading to metal contamination (Adriano, 1986). Chromite ore is most commonly associated with ultramafic rocks, which is very prone to alternation. Serpentinite is generally known as the altered rock, derived from Si poor and Mg rich ultramafic rock by low temperature alteration/metamorphism. Due to the occurrence of chromite, serpentinite derived soil contains abnormally high concentration of Cr which can adversely affect the environment and human health. Chromium released from parent rocks is commonly present in soil as Cr(III) and Cr(VI). The toxicity and mobility of Cr depends on its speciation (McGrath, 1995). The Cr(III) is mostly immobile and considered to be essential trace element for the functioning of living organisms; however Cr(VI) is highly toxic as it is easily soluble and mobile. Concentration of Cr(VI) as low as 5 mg/kg in soil can be toxic to plants (Turner and Rust, 1971). Along with abnormally high concentration of Cr, elevated levels of metals are commonly encountered in soil produced due to various pedogenic processes of serpentinite or ultramafic rock near chromite mines (Krishna et al., 2013; Hseu and Iizuka, 2013; Kanellopoulos et al., 2015).

Soil has an important role in maintaining environmental quality (Beiderbeck et al., 1987). Doran and Parkin (1994) defined the soil quality as it is the capacity of soil to maintain the environment, improve plant and animal health and function properly within ecosystem to provide biological productivity. Mining activities represent the source of potentially toxic metal pollution, which has detrimental effect on the quality of soil. Soil enzymes are important component of soil quality and play vital roles through decomposition of organic matter and nutrient cycling (Castaldi et al., 2004; Angelovičová and Fazekašová, 2014). Soil enzymes are very sensitive to natural and anthropogenic disturbances, and respond very quickly to increased metal concentration (D'Ascoli et al., 2006; Bhattacharya et al., 2008; Hu et al., 2014; Mikanova, 2006). So study of enzyme activities in soil gives useful information on extent of environmental impact of toxic metals. Hinojosa et al. (2004) recommended study of enzymatic activities in soil, to study metal contamination, rather than study of soil physico-chemical properties only as soil enzyme shows significant response to soil pollution. Relatively small amounts (1–10 mg/kg) of Cr(VI) and Cr (25–100 mg/kg) can negatively impact the number, type, health and activity of soil organisms, and so impact the health of the soil ecosystem (Viti et al., 2006). Globally, studies on the influence of chromite mines on soil enzyme activities are very few.

Sukinda ultramafic complex hosts one of the largest chromite deposits in the world and stands 6th in terms of total reserve with nearly 193 million tons, and contributes nearly 95% of the total India's reserve.

* Corresponding author.
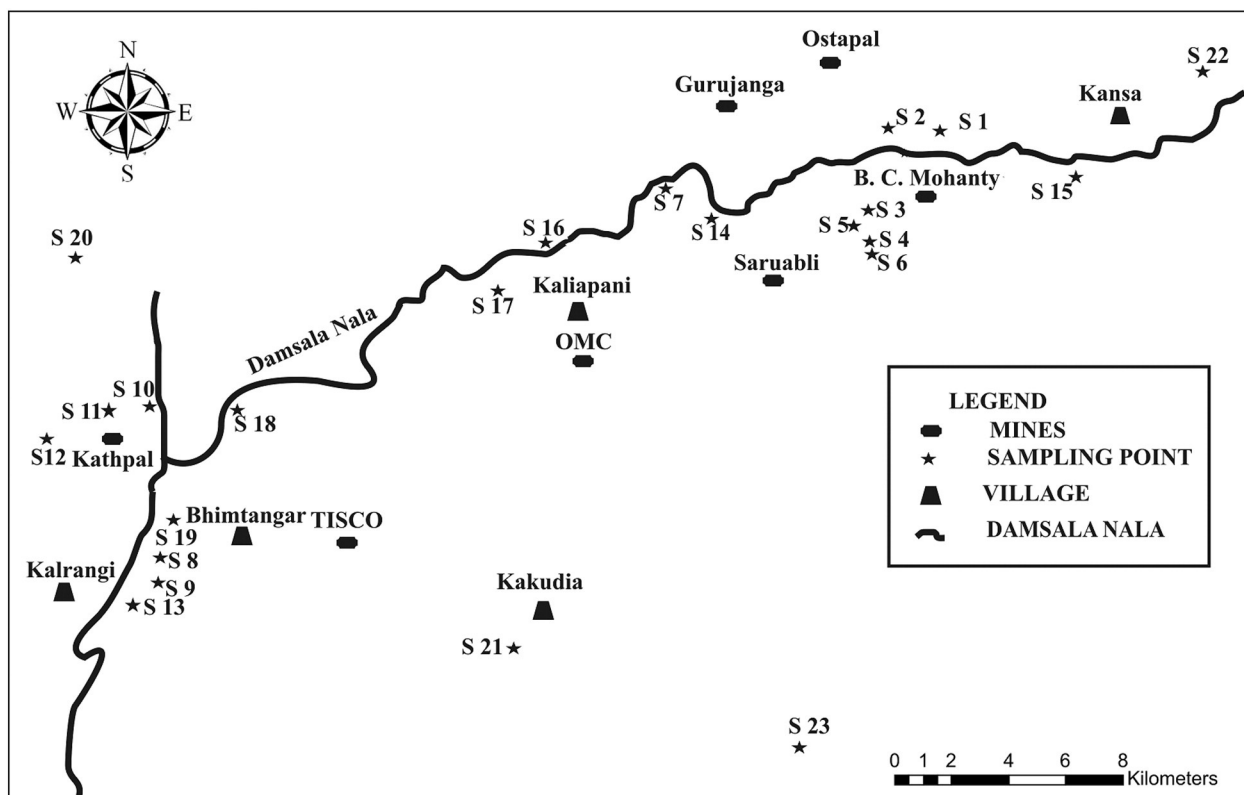E-mail address: md_equeen@yahoo.co.in (Sk. Md. Equeenuddin).

Fig. 1. Soil sample location map in the Sukinda ultramafic complex.

Sukinda ultramafic complex is located in Odisha, eastern part of India. Chromite mining activities in Sukinda valley started since 1960. However-er, in last two decades, the Sukinda valley has drawn lots of attention among scientific communities for the occurrence of high concentration of Cr(VI) in groundwater and surface water (Godgul and Sahu, 1995; Dubey et al., 2001; Dhakate et al., 2008; Tiwary et al., 2005); and various approaches for removal of Cr(VI) from water bodies. The Sukinda valley was identified as one of the most polluted areas in the world due to Cr(VI) pollution in water bodies (Blacksmith Institute, 2007). Das et al. (2013) attempted to study the microbial groups such as fungi, actino-mycetes and bacteria in the mine dumps and nearby soils. However, very few studies carried out on geochemistry of soil, metal contamina-tion and enzyme activities in the Sukinda valley. Therefore, in this paper attempts has been made to study (i) the level of potentially toxic metals in soil and their source, (ii) the influence of metal content on soil enzyme activities.

Table 1
Physicochemical parameters (EC: µS/cm) and metal concentration (mg/kg) in soil unless otherwise mentioned.

Sample	pH	EC	OC (%)	Mn	Cr	Ni	Cu	Zn	Pb	Co	Fe (%)	Cr(VI)
S1	5.38	50.90	0.84	929	960	183	39	56	14	52	2.59	4.35
S2	6.85	32.00	0.41	2324	27,976	3688	48	107	BDL	259	7.79	14.46
S3	7.65	59.30	0.89	3005	49,100	2153	49	131	BDL	316	5.72	16.40
S4	7.40	54.50	1.40	891	5501	1203	42	93	3	120	3.85	6.47
S5	7.33	47.90	1.52	1402	7168	1423	49	98	BDL	142	4.57	6.34
S6	6.57	59.00	1.86	1169	7189	1279	42	85	3	187	3.61	10.88
S7	6.30	36.30	0.35	2889	12,480	1083	50	84	BDL	116	8.4	12.42
S8	6.03	23.76	0.89	4616	9255	791	56	98	58	268	5.83	10.97
S9	6.84	63.00	1.34	5553	9977	1505	44	97	BDL	282	4.83	8.37
S10	5.72	17.78	0.56	387	1139	114	41	53	24	26	2.28	4.14
S11	6.72	29.90	0.49	728	4848	398	70	92	16	54	4.36	4.16
S12	5.71	29.32	0.77	643	959	148	46	57	25	25	2.59	5.44
S13	5.48	21.43	0.74	5445	8145	685	69	93	67	279	6.48	12.92
S14	5.19	23.37	0.55	395	1985	195	31	49	19	42	2.24	4.38
S15	5.83	28.45	1.09	1650	1554	370	49	66	6	102	3.57	5.42
S16	6.01	14.89	0.53	612	1042	143	44	56	26	25	2.12	4.26
S17	6.39	37.90	0.77	860	2078	262	42	58	17	68	2.80	5.98
S18	4.97	22.76	0.62	1557	1517	196	60	74	17	60	3.25	4.53
S19	4.65	188.70	2.04	1317	5546	411	39	72	13	123	3.60	10.13
S20	5.26	26.56	0.68	294	1447	78	31	44	33	16	1.45	4.32
S21	6.07	20.28	1.06	945	703	66	49	64	38	32	2.18	7.74
S22	7.12	55.50	0.58	356	521	58	30	61	23	18	2.03	1.45
S23	5.00	44.90	1.12	284	404	45	26	42	17	12	1.82	2.11

Cl: Clinochlore; T: Talc; P: Plagioclase
Ac: Actinolite; G: Goethite; L: Lizardite
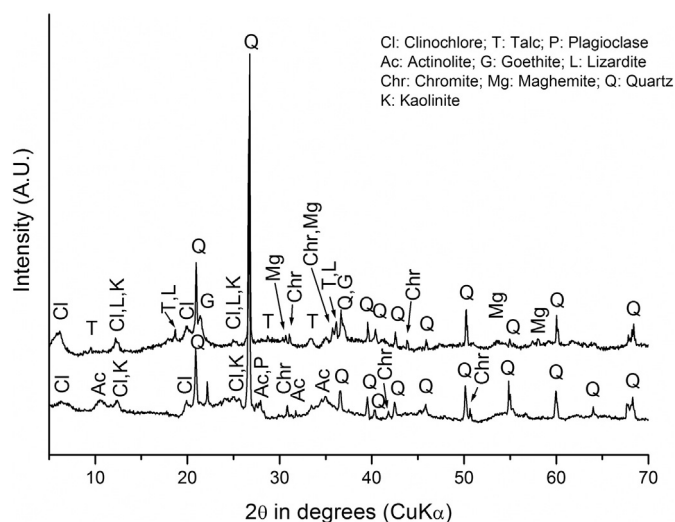Chr: Chromite; Mg: Maghemite; Q: Quartz
K: Kaolinite

Fig. 2. XRD pattern of the soil sample depicting mineralogy.

2. Materials and methods

2.1. Study area

Sukinda ultramafic complex, located in Jajpur district, Odisha which lies in the eastern part of India. It has NE-SW strike and forming a V-shaped valley lying between the northern Daitari hill and southern Mahagiri hill (Das et al., 2013). This ultramafic complex contains significant quantity of stratiform chromite deposit and nickeliferous laterite. It extends for nearly 20 km length, from Kansa in the NE up to Kathapal in SW, with width of approximately 2–5 km. The ultramafic rocks intruded into the Precambrian metamorphites and were emplaced in two phases. Ultramafic body mostly represented by serpentinised dunite-peridotite with chromite bands whereas subordinate amount of pyroxenite devoids of chromite mineralization (Sahoo, 1995). Presently 13 numbers of mines are under operation; out of which 12 practice opencast mining and only one practices underground mining method. The Sukinda valley is mostly dominated by lateritic soil cover. The soil is dominantly sandy in nature with nearly sand (66–84%), slit (9–16%) and clay (7–19%) (Samantaray et al., 2001; Rathore et al., 2014).

The average temperature in the Sukinda valley varies from 15 °C in the month of December to 38 °C in the month of May. The study area is experiencing humid tropical climate and receives average annual rainfall of 1702 mm out of which monsoon season accounts for nearly 80% of total rainfall. Average annual relative humidity and air pressure ranges from 38 to 83% and 982 to 1002 mb respectively.

Table 2
Statistical parameters of soil (Unit of metals: mg/kg; EC: μS/cm).

Parameters	MIN	MAX	MEAN	SD	VAR	SKW	KURT
pH	4.65	7.65	6.13	0.81	0.66	0.16	−0.86
EC	14.89	188.00	42.97	34.35	1179.75	3.24	14.35
OC (%)	0.35	2.04	0.92	0.44	0.20	1.01	0.59
Mn	284.00	5553.17	1663.03	1565.56	2.45E + 06	1.44	1.41
Cr	404.00	49,100.00	7.02E + 03	1.08E + 04	1.16E + 08	2.82	10.06
Ni	45.00	3688.00	716.39	854.34	7.30E + 05	1.96	5.21
Cu	26.00	70.00	45.48	10.91	118.95	0.48	0.48
Zn	42.00	131.00	75.22	22.62	511.82	0.49	−0.29
Pb	0.00	67.00	18.22	17.52	307.04	1.23	1.79
Co	12.00	316.00	114.09	98.81	9763.73	0.81	−0.70
Fe (%)	1.45	8.39	3.82	1.88	3.53	0.94	0.31
Cr(VI)	1.45	16.40	7.29	3.97	15.73	0.71	−0.34

MIN: Minimum, MAX: Maximum, SD: Standard Deviation, VAR: Variance, SKW: Skewness, KURT: Kurtosis.

2.2. Sampling and analysis

Twenty soil samples were collected from paddy fields within a depth of 15 cm in the Sukinda valley (Fig. 1) and three samples (S20, S22 and S23) were collected far away from the study site, as baseline soil, which are considered as controlled sample. Nearly 1 kg of soil sample was collected with a hand spade, by removing upper vegetation of soil, from each location. Samples were stored in cleaned and sealed polythene bag for further analysis. Soil samples were dried at room temperature. After homogenization all the samples were manually and gently powdered and sieved through 200 mesh and stored in cleaned and sealed polythene bag.

Physicochemical parameters of soil were analysed on air dried powdered sample. Soil pH and electrical conductivity were measured in soil: water (deionized) ratio of 1:2.5 and 1:5 respectively by using Orion Ion selective electrodes from <2 mm soil fraction. Organic carbon content was determined by following wet oxidation method (Walkley and Black, 1934). The detailed mineralogy of soil samples was studied from the powdered samples (<63 μm) by UV-Ultima (IV) X-ray diffractometer (XRD) using CuKα radiation. Major elements and trace metals were determined by wave dispersive X-ray fluorescence spectrometer (Model- Bruker S8 Tiger) by using press pallet. The precision for XRF analysis was within ± 5% for all metals; however, accuracy was within ± 5% for major elements and ± 10% for trace elements. Total Cr(VI) in soil was determined by following heated carbonate-hydroxide extraction method (James et al., 1995). Morphology of soil samples was observed under Field Emission Scanning Electron Microscope (FESEM) (Carl Zeiss SUPRA 40) operated at 3 kV and chemical composition was
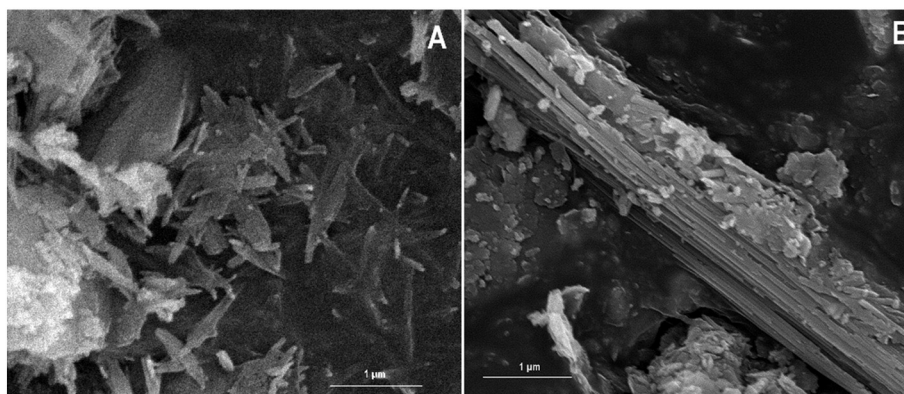

Fig. 3. FESEM microphotograph showing lizardite (A) and actinolite (B).

observed using EDX analysis, which is attached to FESEM system. For the FESEM study, samples were coated with a thin film of gold with help of a Polaron sputter coater.

Fresh moist soil samples were used for enzyme analysis. Soil enzymes such as urease activities, acid and alkaline phosphatase, dehydrogenase, and β-glucosidase were estimated following the methods suggested by Tabatabai and Bremner (1972), Eivazi and Tabatabai

(1977), Casida et al. (1964) and Eivazi and Tabatabai (1988) respectively. Enzyme activities were determined in triplicate with error <5%.

Statistical evaluation of analytical data was conducted to generate statistical parameters, which is required for interpretation of data. Skewness and kurtosis were calculated to study the normality of data distribution. The boxplot and the principal component analysis were carried out by using SPSS version 22. Descriptive statistical parameters
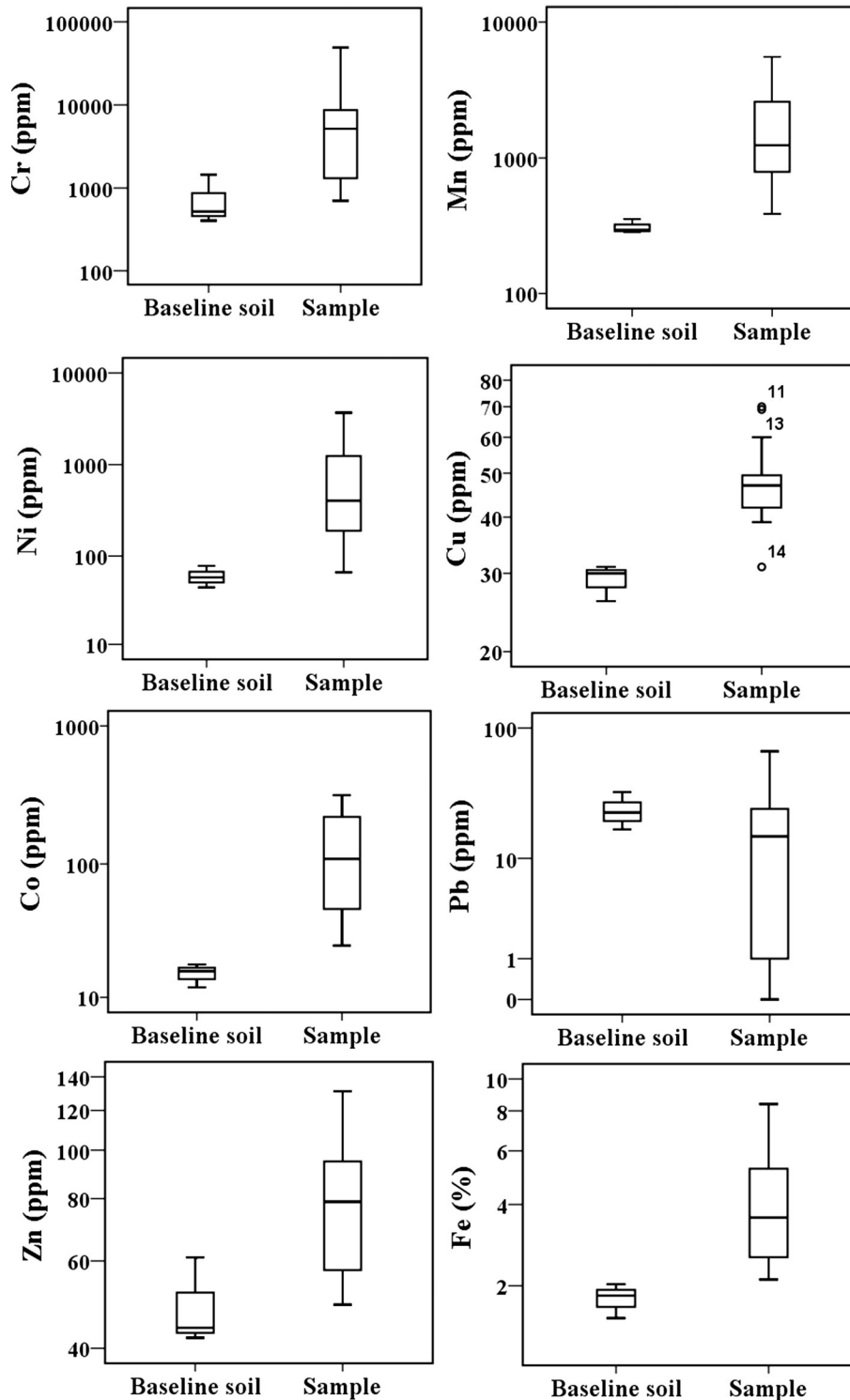

Fig. 4. Comparison of total metal concentration in baseline and nearby mines soil.

like mean, median, variance, standard deviation along with skewness, kurtosis and Pearson correlation coefficient were determined by Microsoft Excel 2016.

3. Result and discussion

3.1. Soil physicochemical properties and mineralogy

Soil physicochemical parameters are given in Table 1. Soil is acidic to slightly alkaline in character with its pH varies from 4.65 to 7.65. Organic carbon of the study area is very low, ranges from 0.35 to 2.04% with an average 0.92%. Electrical conductivity ranges from 14.89 to 188.70 μs/cm; with an average 42.29 μs/cm.

The XRD spectrum of the soil sample (Fig. 2) shows the dominance of quartz in all the samples. Beside quartz, common soil mineral phases comprise of clinochlore, lizardite, talc, actinolite, illite, goethite, chromite and maghemite with their abundance varies from trace to moderate amount. The FESEM microphotographs also clearly indicate the occurrence of lizardite and actinolite (Fig. 3). These minerals are most commonly derived weathered product of the ultramafic rocks.

3.2. Major and trace element concentration

The average concentration of MgO, CaO, Fe_2O_3 and Al_2O_3 in soil is 1.58 wt%, 0.58 wt%, 11.45 wt% and 14.09 wt% respectively. However, in the parent rock average concentration of MgO, CaO, Fe_2O_3 and Al_2O_3 is 29.3 wt%, 1.6 wt%, 8.22 wt%, 0.53 wt% respectively. Lower concentration of MgO and CaO in the soil in comparison to that of the ultramafic rock indicates that it is due to their leaching during rock weathering and soil formation. However, higher concentration of Fe_2O_3 and Al_2O_3 in soil than that of ultramafic rock is due to their relatively lower mobility which leads to the accumulation of Fe and Al oxyhydroxides. Although typical serpentinite soil is characterized by high concentration of MgO; but in the Sukinda valley soil has relatively low concentration of MgO. This is mostly due to the extensive leaching of the parent rocks, which is evident by the presence of thick lateritic cover in the field. Som and Joshi (2002) reported that Mg was completely leached from the laterite and saprolite.

Potentially toxic metal concentration in soil and their statistical analysis are given in Table 1 and Table 2. Concentration of Cr ranges from 703 to 49,100 mg/kg having an average of 7646 mg/kg, Ni varies from 66 to 3688 mg/kg with mean value of 780 mg/kg, Mn concentration varies from 294 to 5553 mg/kg with an average of 1791 mg/kg. Concentrations of Cu, Co and Zn range from 31 to 70 mg/kg, 16 to 316 mg/kg and 44 to 131 mg/kg respectively with an average of 47.14 mg/kg, 123.52 mg/kg, 77.48 mg/kg respectively. Concentration of Pb ranges up to 58 mg/kg with an average of 18 mg/kg. Concentration of total Cr(VI) in soil varies from 1.45 to 16.7 mg/kg with an average of 7.3 mg/kg. Due to prevalence of ultramafic rocks in the study area Cr, Mn and Ni concentration are very high in soil samples. Dermatas et al. (2015) reported 5.33 mg/kg of average Cr (VI) in top soil, over ophiolite, having range from 4.0 to 7.5 mg/kg in Vergina of northern Greece. Kien et al. (2010) observed 9.79 mg/kg of Cr (VI) in paddy soil near the

chromite mine in Vietnam and 1.25 mg/kg of Cr (VI) in uncontaminated soil. High level of Cr(VI) in soil is mostly caused through oxidation reaction by Mn-oxides (Oze et al., 2004). Das et al. (1996) reported the presence of minor amount of Mn-oxides such as lithiophorite and chalcophanite in the chromite ore. Also the concentration of Mn in the soil of study area is very high. Comparison of potentially toxic metal concentration in soil with uncontaminated baseline soil is shown in Fig. 4. It is observed that except Pb, concentrations of all the metals are greater than that of the baseline soil. The average concentration of Cr in soil is nearly 84 mg/kg worldwide (McGrath, 1995), whereas Cr concentration of 12.5 wt% has been reported in serpentinite derived soil (Adriano, 1986). Hsiao et al. (2009) reported that Co content in the serpentinitic soils from eastern Taiwan was up to 239 mg/kg. Concentration of Fe is very high in and around chromite mines and its vicinity as the ultramafic rock hosting chromite is very prone to weathering and resulting in the thick lateritic cover while away from the mining areas, Fe concentration is very less. Trace metals concentration in the soil developed over the ultramafic rocks in various parts of the world is given in Table 3. It is observed that very high concentration of Cr, Ni, Mn and Co are present in serpentinite soil similar to that of Sukinda valley and the most of the elements are within the range (Pb, which are just marginally higher) to that of the global occurrence. Elevated level of Cr, Ni and Co in soil is mainly known to be derived from the weathering of ultramafic rocks (Kanellopoulos et al., 2015). Based on Indian safety limit (Table 3), it can be concluded that concentration of Co and Ni are well above the safe limit of Indian standard, and safe limit for Cr and Mn are not available as per Indian limit.

3.3. Soil toxicity

Level of soil toxicity has been evaluated using various commonly used parameters such as enrichment factor (EF), geoaccumulation index (I_{geo}), pollution index (PI) and contamination index (CI). Enrichment factor is a convenient measure of geochemical trend and is used to measure the enrichment of elements with respect to their crustal abundance. Generally, normalization of metal concentration with conservative element like Al has been used in calculation of EF to study the anthropogenic impact on soil. This normalization compensates the natural variability caused due to the influence of grain size and mineral composition on elemental concentration. Following equation was used to calculate enrichment factor (Diop et al., 2015).

$$EF = [Me/Al]_{Soil}/[Me/Al]_{bg}$$

Here $[Me/Al]_{soil}$ is the metal to Al ratio in the sample and $[Me/Al]_{bg}$ is the metal to Al ratio in the upper crustal materials. Average crustal abundance of Al (7.96 wt%), Cr (126 mg/kg), Co (24 mg/kg), Cu (25 mg/kg), Mn (716 mg/kg), Ni (56 mg/kg), Zn (98 mg/kg) and Pb (14.8 mg/kg) were used from Wedepohl (1995). The EF in the Sukinda ultramafic complex is in the order of Cr > Ni > Co > Mn > Cu > Zn > Pb (Fig. 5). Based on the degree of metal pollution in soil (Table 4), there is extremely severe enrichment, severe enrichment and moderately

Table 3
Comparison of metal content in soil over ultramafic rocks with that of Sukinda and Indian safety limit (Unit: mg/kg).

Location	Co	Cr	Cu	Mn	Ni	Pb	Zn	Reference
Atalanti, Greece	28	213	54	787	260	16.0	68	Kanellopoulos et al. (2015)
Ussangoda, Sri Lanka	157	11,301	30	1117	6776	NA	173	Rajapaksha et al. (2012)
Nuggihalli, India	22	500	24	NA	168	11.3	99	Krishna et al. (2013)
Morais Massif Portugal	NA	3400	31	2500	3166	NA	91	Alves et al. (2011)
Kroondal 3049 IQ South Africa	NA	489	27	NA	307	6.3	22	Gzik et al. (2003)
New Caledonia French island	NA	8893	NA	4423	8376	NA	NA	Becquer et al. (2003)
Sukinda, Odisha	124	7646	47	1791	780	18.1	77	This study
Indian Safety Limit	60–100	NA	135–270	NA	75–150	250–500	300–600	Awasthi (2000)

NA: not available.

severe enrichment for Cr, Ni and Co respectively. However, there is no to minor enrichment for other metals.

Geoaccumulation index is used to assess the soil contamination by comparing the concentration of toxic metal in soil with that of uncontaminated soil. The geoaccumulation index is calculated using the following formula (Ruiz, 2001):

$I_{geo} = \log_2(^{Cn}/_{1.5Bn})$; where Cn is the measured concentration of element present in the sample, Bn is the concentration of element in uncontaminated soil and the constant 1.5 is used to study the natural variation of the element in the sample with anthropogenic disturbances. Soils have been classified into 6 classes based on the I_{geo} and

Fig. 5. Variation of enrichment factor, geoaccumulation index, pollution and contamination index.

Table 4
Enrichment Factor (Sutherland, 2000).

Levels	Enrichment factor
<2	Depletion to minimal enrichment suggestive for or minimal pollution
2–5	Moderate enrichment, suggestive of moderate pollution
5–20	Significant enrichment, suggestive of a significant pollution signal
20–40	Very highly enriched, indicating a very strong pollution signal
>40	Extremely enriched, indicating an extreme pollution signal

contamination level is shown in Table 5. Based on the I_{geo} most of the samples in the study area can be classified into strongly contaminated by Cr, Ni and Co; whereas other elements are categorized into uncontaminated to moderately contaminated (Fig. 5).

The pollution index (PI) is used in this study to identify the multi-element contamination potential and was calculated by averaging the ratio of metal concentrations in samples to their assumed permissible levels. The tolerance limits suggested by Kabata-Pendias and Pendias (2001) were adopted for Co (50 mg/kg), Cr (100 mg/kg), Cu (100 mg/kg), Mn (300 mg/kg), Ni (100 mg/kg), Pb (100 mg/kg) and Zn (300 mg/kg).

$$PI = \frac{^{Co}/_{50} + ^{Cr}/_{100} + ^{Cu}/_{100} + ^{Mn}/_{300} + ^{Ni}/_{100} + ^{Pb}/_{100} + ^{Zn}/_{300}}{7}$$

PI of soil ranges from 1.8 to 75 (average 13.3) (Fig. 5). PI > 1 indicates concentrations of metals are above the permissible levels and hence, is considered as phytotoxic.

Enrichment of any element to an excessive degree is harmful to the environment. The cumulative enrichment factor of elements is often more meaningful for environmental studies than that of an individual metal contaminant (Prusty et al., 1994). Hence, metal concentrations obtained for each sample point was reduced to one parameter known as "contamination index" (CI). The level of contamination of an element is measured with reference to its background concentration. The CI was calculated following Rang et al. (1987):

$$CI = \sum_{N=1}^{n} (C_N/B_N)$$

where, C_N = concentration of Nth element, B = background value for Nth element, n = number of elements (7). CI in soils varies between 8.6 and 183 (Fig. 5). This indicates that soil in the Sukinda ultramafic complex has been are highly contaminated and the level of contamination is specifically much higher in areas close to the mines.

3.4. Soil enzyme activities

Enzyme activities in soil are considered as an ecological indicator of soil quality (Alkorta et al., 2003; Hu et al., 2014). Variation of enzyme activities in the Sukinda valley in comparison to that of control soil is shown in Fig. 6. Soil urease activity varies from 104.67 to 211.89 μg urea/g/h, with an average 141.96 μg urea/g/h. Mean value of
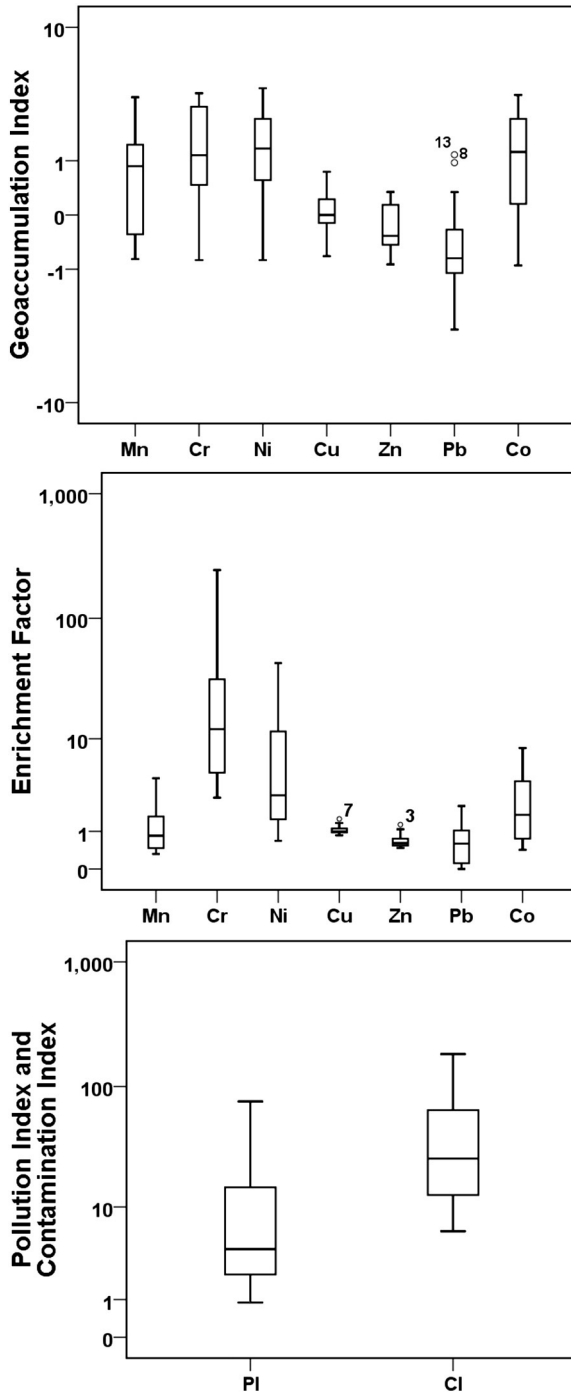
Table 5
Index of geoaccumulation and contamination level (Muller, 1969).

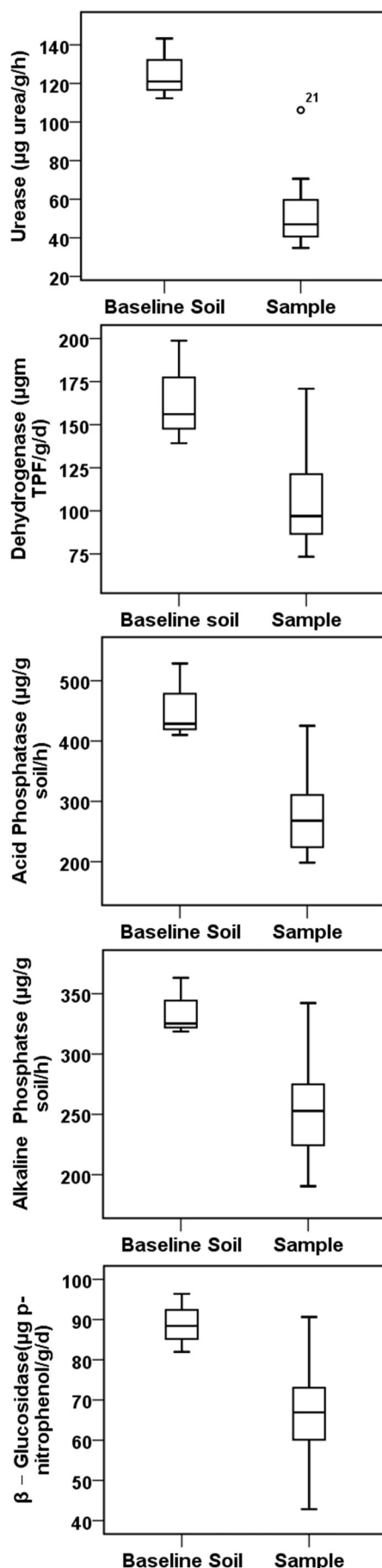| I_{geo}	Category	Contamination level
<0	0	Uncontaminated
0–1	1	Uncontaminated to moderately contaminated
1–2	2	Moderately contaminated
2–3	3	Moderately to strongly contaminated
3–4	4	Strongly contaminated
4–5	5	Strongly to extremely contaminated
>5	6	Extremely contaminated

Fig. 6. Variation of enzyme activities in baseline and nearby mines soil.

soil dehydrogenase is 278.101 µg TPF/g/d and it ranges from 174.65 to 429.54 µg TPF/g/d. Acid phosphatase activity varies from 0.57 to 1.38 µg/g soil/h with average of 1.02 µg/g soil/h. Alkaline phosphatase activity varies from 0.43 to 1.48 µg/g soil/h, with mean value of 0.95 µg/g soil/h. β-glucosidase activity in soil of the study area varies from 85.16 to 227.91 µg p-nitrophenol/g/d, with average of 150.83 µg p-nitrophenol/g/d.

Correlation matrix of soil physicochemical parameter, potentially toxic metal concentration and enzyme activities is given in Table 6. In present study, soil pH is positively significantly correlated with most of the metals except Mn, Cu and Pb. It is may be due to co-precipitation of metals along with formation of Fe-oxyhydroxides with increase in pH and subsequent adsorption onto Fe-oxyhydroxides, which has shown high scavenging potential for metals. Organic carbon of study area does not show any significant relationship with soil enzyme. Low amount of organic carbon in soil cannot be significantly correlated with soil enzymes (Wang et al., 2007).

Unlike metals, pH shows negative correlation with all the soil enzymes. Zhang et al. (2008) described dehydrogenase enzyme, which plays an important role in soil ecosystem through biological oxidation of soil organic carbon and transfers the hydrogen ion from organic substrates to inorganic substances. Dehydrogenase is most sensitive to metal pollution (Mikanova, 2006; Wyszkowska et al., 2001; Peng et al., 2009) and it is considered as an indicator of microbial activities affected by toxic metals. In present study dehydrogenase activity is negatively correlated to Ni ($r = -0.61$), Co ($r = -0.71$), Cr ($r = -0.53$), Zn ($r = -0.74$), Mn ($r = -0.55$), Cu (-0.48) and Fe ($r = -0.71$). Wyszkowska et al. (2006) observed that about 50 mg/kg of metals (Cu, Zn, Ni, Pb, Cd and Cr) reduced dehydrogenase activity significantly in soil. Optimum range of pH for dehydrogenase activity is around 7.4 to 8.9 (Nagatsuka and Furosaka, 1980), but pH of most of the soil samples in the study area varies from 4.65 to 7.65. It suggests soil pH is also not conducive for dehydrogenase activity in Sukinda valley. Peng et al. (2009) explained dehydrogenase activity as an indicator of chromium pollution in soil. Wyszkowska et al. (2001) described impact of Cr(VI) on enzyme activities. Increase of chromium dose ($K_2Cr_2O_7$) from 40 mg/kg to 120 mg/kg caused decrease in dehydrogenase from 2.90 mm³ H_2 to 0.25 mm³ H_2.

Urease is an endoenzyme, and catalyzes the hydrolysis of urea, which is the source of nitrogen for plant (Kiss et al., 1975). In present study it is found that Cr ($r = -0.40$), Ni ($r = -0.52$), Zn ($r = -0.59$), Mn ($r = -0.41$), Cu ($r = -0.51$), Co ($r = -0.54$) and Fe ($r = -0.60$) have negative correlation with soil urease activity. Samborska et al. (2004) conducted experiment by exposing urease to Cr(III) and Cr(VI) dose. They observed ED_{50} value of Cr(III) is much lesser than Cr(VI). It indicates Cr(III) is much harmful than Cr(VI) in case of soil urease activity. Wyszkowska et al. (2006) arranged the metals in order of the toxicity to urease enzyme in the following order as Cr (VI) > Zn > Pb > Cu > Ni.

β-glucosidase is an extracellular enzyme, which helps in the degradation of cellulose of plant cell (Turner et al., 2002). These enzymes are sensitive to environmental stress and possess microbial ecological significance (Yakovchenko et al., 1996). β-glucosidase actively participate in organic matter cycling, which is considered as major component of soil quality and an important parameter of organic matter turnover (Debosz et al., 1999). Turner et al. (2002) and Hinojosa et al. (2004) showed β-glucosidase as a decisive indicator of soil contamination by toxic metals. In present study β-glucosidase is negatively significantly correlated with metals such as Mn ($r = -0.44$), Cr ($r = -0.66$), Ni ($r = -0.80$), Cu ($r = -0.42$), Zn ($r = -0.79$), Co ($r = -0.71$) and Fe ($r = -0.75$). Geiger et al. (1998) described the inhibitory effect of Cu, Zn and Ni on β-glucosidase activity. Kuperman and Carreiro (1997) described significant depression of 69–96% in case of β-glucosidase due to exposure of heavy metals. Kucharski et al. (2009) conducted a field based experiment and found that there is 9% reduction of β-glucosidase due to exposure of Ni.

Table 6

Pearson correlation coefficient among various parameters.

	pH	EC	OC	URE	AP	ALP	B-GL	DEH	Mn	Cr	Ni	Cu	Zn	Pb	Co	Fe	Cr (VI)
pH	1.00																
EC	−0.10	1.00															
OC	0.05	0.70*	1.00														
URE	−0.24	−0.05	−0.10	1.00													
AP	−0.39**	−0.15	−0.19	0.86*	1.00												
ALP	−0.32	−0.19	−0.05	0.77*	0.91*	1.00											
B-GL	−0.41**	−0.21	−0.17	0.78*	0.91*	0.89*	1.00										
DEH	−0.36**	−0.27	−0.17	0.77*	0.93*	0.90*	0.87*	1.00									
Mn	0.16	0.01	0.06	−0.41**	−0.52*	−0.60*	−0.44*	−0.55*	1.00								
Cr	0.52*	0.12	−0.04	−0.39**	−0.51*	−0.65*	−0.66*	−0.53*	0.43*	1.00							
Ni	0.59*	0.09	0.07	−0.52*	−0.66*	−0.68*	−0.80*	−0.61*	0.44*	0.79*	1.00						
Cu	0.12	−0.24	−0.18	−0.51*	−0.42*	−0.40**	−0.42*	−0.48*	0.52*	0.20	0.20	1.00					
Zn	0.65*	0.13	0.16	−0.59*	−0.72*	−0.75*	−0.79*	−0.74*	0.65*	0.79*	0.79*	0.58*	1.00				
Pb	−0.43*	−0.31	−0.25	0.27	0.35	0.29	0.43*	0.34	0.23	−0.32	−0.45*	0.26	−0.24	1.00			
Co	0.41**	0.16	0.23	−0.54*	−0.70*	−0.76*	−0.71*	−0.71*	0.85*	0.73*	0.76*	0.42*	0.87*	−0.04	1.00		
Fe	0.37**	0.02	−0.11	−0.60*	−0.69*	−0.75*	−0.75*	−0.71*	0.72*	0.64*	0.74*	0.57*	0.79*	−0.14	0.77*	1.00	
Cr(VI)	0.27	0.18	0.14	−0.60*	−0.70*	−0.74*	−0.74*	−0.70*	0.66*	0.77*	0.73*	0.45*	0.78*	−0.06	0.84*	0.81*	1.00

URE: Urease; AP: Acid Phosphatase; ALP: Alkaline Phosphatase; B-GL: β- Glucosidase; DEH: Dehydrogenase.
 * Correlation is significant at the 0.05 level.
 ** Correlation is significant at the 0.10 level.

Phosphatase acts as catalyst in hydrolysis of ester and anhydride of phosphoric acid (Schmidt and Laskowski, 1961). It is observed that acid phosphatase and alkaline phosphatase are negatively correlated to all the metals except Pb. Wyszkowska et al. (2001) described inhibitory impact of chromium on soil acid phosphatase and alkaline phosphatase activity. Yang et al. (2006) described stimulatory effect of Pb on soil enzymatic activities and acts as protective element of enzymes up to certain limit. This justifies the positive correlation of all soil enzymes with Pb. Xia et al. (2002) observed positive correlation between Pb and soil enzymes as Pb acted as stimulator for soil enzymes as present in very low concentration in soil solution. In Sukinda valley concentration of Pb is very low, and varies from BDL to 67 mg/kg.

Metals sensitivity to enzyme activities have been studied and reported in many literatures. Copper, Pb, Zn and Cd are the most commonly studied metals in relation to enzyme activities. However, few studies have been carried out on impact of very high concentration of Cr, Cr(VI) and Ni. Most of the studies reported that dehydrogenase and urease are the most sensitive enzyme to be impacted by metals. Hu et al. (2014) reported that declined activity of urease was very high which is followed by dehydrogenase and acid phosphatase in comparison to that of control soil due to more concentration of Cu, Zn and Cd in the paddy field. It is observed that reduction in the percentage of enzymatic activities in comparison to control soil are 58.54%, 40.56%, 24.25%, 27.88% and 37.10% for urease, acid phosphatase, alkaline phosphatase, β-glucosidase and dehydrogenase respectively. Thus, in this study enzyme activities are reduced in the following order: urease > acid phosphatase ≥ dehydrogenase > β-glucosidase ≥ alkaline phosphatase, which indicates that there is maximum reduction of urease activity in the Sukinda valley (Fig. 6). Shen et al. (2005) reported that order of inhibition of urease activity generally decreases according to sequence Cr > Zn > Mn > Pb. Kucharski et al. (2009) showed decreasing order of sensitivity of enzyme due to nickel contamination as urease > dehydrogenases > alkaline phosphatase > acid phosphatase > β-glucosidase. Due to more sensitivity of urease among the studied enzymes in soil, it can be regarded as the indicator of soil environmental quality.

3.5. Factor analysis

Factor analysis was performed on the dataset of soil for Cr, Cr(VI), Co, Fe, Cu, Mn, Ni, Pb Zn, and all enzyme activity using the Principal Component analysis for understanding their controlling factors. Three factors were extracted corresponding to eigen value >1 and they explain 86.14% of the total variance. Varimax rotated factor loading are tabulated in Table 7. Factor loadings are graphical shown in a 3D plot (Fig. 7). For interpreting a group of variables associated with a particular factor, loading >0.6 was considered. The first factor (Factor-1) describes 65.1% of the variance and is highly and positively loaded with all the metals except Pb and Cu. This indicates that Cr, Cr(VI), Ni, Zn and Co is probably associated with Fe- and Mn-oxyhydroxides, and derived from same source. The EDX spectra also indicated the presence of high concentrations of Cr, Ni and Mn in goethite. The second component (Factor-2) describes 12.4% of the variance and is highly loaded with all enzymes. Factor-3 (8.6%) includes only Cu and Pb suggesting a different source and behavior for these metals. It is also clearly visible that enzyme activities are negatively correlated related with metals except Pb (Table 6). Some of the studies also reported that higher concentration of metals cannot negatively impact the activities of enzymes in soil due to availability of high OC (D'Ascoli et al., 2006; Tripathy et al., 2014). High OC in soil acts like a protective cover for enzymes by precipitating potentially toxic metals as insoluble complex and forms enzyme-humus complex, which protects the activities of soil enzymes from metal contamination. However, as the soil OC is very low in the Sukinda valley, metals, except Pb, have impacted negatively to activities of dehydrogenase, urease, acid phosphatase, alkaline phosphatase and β-glucosidase.

Table 7

Factor loading for enzymes and various metals.

Metals	Factor 1	Factor 2	Factor 3
UR	−0.216	0.892	−0.068
AP	−0.399	0.882	0.013
ALP	−0.546	0.759	−0.018
GL	−0.547	0.784	0.128
DEH	−0.421	0.845	−0.038
Fe	0.703	−0.507	0.238
Zn	0.773	−0.406	0.127
Cr	0.890	−0.200	−0.165
Mn	0.626	−0.277	0.515
Ni	0.817	−0.380	−0.250
Cu	0.166	−0.508	0.671
Pb	−0.148	0.366	0.836
Co	0.838	−0.379	0.261
Cr(VI)	0.838	−0.350	0.178
% Total Variance	65.1	12.4	8.6
Eigen Value	9.1	1.7	1.2

UR: Urease; AP: Acid Phosphatase; ALP: Alkaline Phosphatase; GL: β- Glucosidase; DEH: Dehydrogenase.
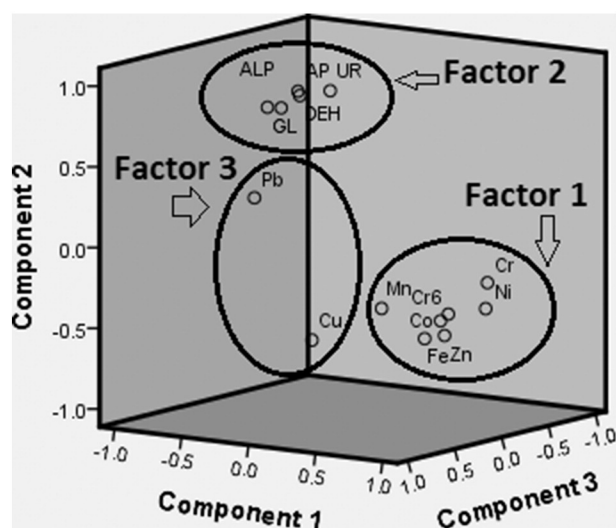
Fig. 7. Factor loading pattern of metals and enzyme activities.

4. Conclusions

The soil in the Sukinda valley has pH 4.65 to 7.65, organic carbon content varies from 0.35 to 2.04% with an average 0.92%. Concentration of Cr ranges from 703 to 49,100 mg/kg followed by Mn (294–5553 mg/kg), Ni (66–3688 mg/kg), Co (16–316 mg/kg), Zn (44–131 mg/kg), Cu (31–70 mg/kg) and Pb up to 58 mg/kg. Concentration of total Cr(VI) in soil varies from 1.45 to 16.7 mg/kg with an average of 7.3 mg/kg. The enrichment factor with respect to crustal abundance in the Sukinda ultramafic complex is in the order of Cr > Ni > Co > Mn > Cu > Zn > Pb. Based on the I_{geo} most of the samples in the study area are classified into strongly contaminated by Cr, Ni and Co. Both pollution and contamination index suggest that soil near the mines are severely contaminated by metals. Enzyme activities are lower in soil near the mines in comparison to that of uncontaminated soil. Reduction in the percentage of enzymatic activities in comparison to control soil are 58.54%, 40.56%, 24.25%, 27.88% and 37.10% for urease, acid phosphatase, alkaline phosphatase, β-glucosidase and dehydrogenase respectively which indicates that inhibition of urease activities is maximum near chromite mines.

Acknowledgements

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