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Original Research Article

Phosphatase Activity and Heavy Metal Distribution in Soils of Vellore, India

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Abstract

The present work reports the biogeochemical analysis of the soil quality in Vellore which is considered as industrial hotspot of Tamil Nadu, India. The industrial area dominates with leather industries and is highly contaminated with heavy metals. In this study, the enzyme activities and other important physico-chemical properties were estimated in specific locations of the in and around the city. The work also gives an estimate of using enzyme activities as an important indicator of soil quality. The data indicates that the phosphorus in the soil was unavailable for microorganisms due to presence of very high concentrations of heavy metals, which was observed indirectly from low microbial biomass and high bioavailable phosphorus.

Keywords

Heavy metals
Phosphatase activity
Phosphomonoesterases
Soil phosphorus
Soil pollution

Introduction

Soil attributes naturally vary over time; precipitation and temperature influence biological activity, microbial consortium contributed by the seasonal fluctuations (Bandick and Dick, 1999). Biological processes respond more sensitively to environmental changes than chemical and physical properties. Thus, the fluctuations in biochemical pathways can have major, long-lasting effects on organic matter breakdown and nutrient release in the soil (Edwards et al., 1996). Soil biochemical properties are potential indicators of soil quality and these properties can be utilized both individually, as simpler indices, or in combination, using complex equations derived from mathematical combinations or the application of statistical programs. One potentially important soil attribute that may be efficient indicator for soil quality is enzyme activity (Saviozzi et al., 2001) since it is an integral part of life processes occurring within the soil.

Phosphorus is an essential nutrient. In spite of its wide distribution in nature, P is a limited resource (Shimamura et al., 2003) and it is deficient in most soils (Vassilev et al., 2001). Extracellular phosphatases are of interest for their role in mineralizing P. Studies indicate that high enzyme activity signals element limitation in the ecosystem (Ndakidemi, 2006). Soil acid phosphatases constitute an important link between biologically unavailable and bioavailable P pools in the soil (Speir and Ross, 1978). Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick and Tabatai, 1992; Dick et al., 2000).

The strong inhibition activities of a variety of enzymes have been reported in metal polluted soils over the past few years (Huang and Shindo, 2000). However, little is known about the phosphomonoesterase activities and soil quality in significant locations of Vellore, India. Vellore and Ranipet are considered as industrial hub of Tamil Nadu, India, with the cluster of leather industries. The effluent from the industries is the primary source of heavy metal contamination. The aim of this study is to determine the distribution of heavy metals and phosphomonoesterase activity along with important physico-chemical properties of soil in significant locations of Vellore city for comparison and to assess soil quality in the industrial area of Vellore.

Materials and methods

Determination of physico-chemical properties

Soil pH was measured using glass electrode. Electrical conductivity was measured using Conductivity Meter (Systronics Conductivity Meter 304) at 26±1^{o}C. $NaHCO_3$–extractable N was estimated according to Fox and Piekielek (1978). Microbial biomass was estimated by the pour plate technique. Total phosphorus concentration was estimated according to Kopacek and Hejzlar (1995).

Soil organic matter was determined by according to Schumacher (2002). The percentage of soil organic carbon is calculated according to Bowman (1997), by using the formula:

SOM / 1.7 = Soil Organic Carbon (SOC) content

Determination of heavy metals

The concentrations of Cu, Zn, Pb, Cr, Ni, Co, Mg, Mn and Fe was analysed suing Atomic Absorption Spectrometer (VARIAN AA240, Austria) according to Abida et al. (2008).

Phosphatase activity

Acid and Alkaline Phosphatase (EC 3.1.3.2., EC 3.1.3.1.) activities were determined according to Bowman, (1997). Reaction mixtures contained 500 µL of 25 mM p-nitrophenylphosphate (pNPP) in 50 mM sodium acetate buffer (pH 5.0) and in 100 mM Tris buffer (pH 8.5) or in water and 50 mg wet soil sample. The absorbance was read using UV-Visible Spectrophotometer at 405 nm.

Estimation of microbial biomass

Microbial biomass was estimated using spread plate method. One gram of soil was added to the 10 ml DW and it was serially diluted. 100 µl from appropriate dilution was taken and inoculated on the nutrient agar medium and incubated the plates at 37ºC for bacteria and room temperature for fungus. The colonies were counted and results were reported in Colony Forming Units (CFU)

Statistical analysis

One way ANOVA is performed between the heavy metal concentrations, soil phosphorus and phosphatase activities at $p \leq 0.05$ and $p \leq 0.001$ where ever necessary. Relationship between data was tested using Pearson's correlation coefficient. All analyses were performed using BioStat 2008 (v5.2.5).

Results and discussion

Phosphorus content and phosphatase activity

The total Phosphorus concentration was highest in VIT University compared to other locations in Vellore which is shown in Table 1. Phosphatase activity is considered as a reliable indicator of phosphorus limitation in the soil (Tarafdar and Jungk, 1987; Goldstein, 1992; Duff et al., 1994; del Pozo et al., 1999; Haran et al., 2000; Baldwin et al., 2001; Miller et al., 2001; Li et al., 2002). Dracup et al. (1984) reported that increase in phosphatase activity was also seen in cell walls of roots when there is a phosphorus deficiency. The values observed are much higher than those reported by Qureshi et al. (2001) in Pakistan. Phosphorus concentrations of Vellore city (225.0 mg Kg^{-1}) and Amrithi forest (241.5 mg Kg^{-1}) were comparable to VIT University. Acid and alkaline phosphatase activities were given in the Fig. 1.

Table 1. Soil physical parameters of study locations.

Parameters	Ranipet	Bramhapuram	Vellore City	VIT University	Palar Basin	Amirthi Forest
Soil pH	8.03	8.12	8.10	7.04	7.35	8.03
Electrical Conductivity*	1.02	0.46	0.33	0.32	0.24	0.27
Total Phosphorus (P_T)**	40.2	158.9	225	250.6	198.2	241.5
Inorganic Phosphorus (P_i)**	28.0	45.0	47.5	49.0	35.0	48.5
Organic Phosphorus (P_o)**	12.2	113.0	177.5	201.6	163.2	193.5
$NaHCO_3$ Extractable Nitrogen**	0.62	0.93	0.79	0.83	0.29	1.03

* Values are expressed in dS m^{-1}; ** Values are expressed in mg Kg^{-1} Soil

Acid phosphatase activity was observed to be lowest in VIT University. This may be due to the inhibition of enzyme activity by high metal concentrations. But the metal concentrations at Amrithi forest was ineffective on the acid phosphatase activity because forest soil have found to have highest mean acid phosphatase activity and this could be probably explained by the unavailability of the bioavailable phosphorus in these sites.

The enzyme levels in soil systems vary in concentrations primarily due to the soil type which has different amounts of organic and inorganic phosphorus, soil organic content, soil moisture, composition and activity of its living organisms and intensity of the biological processes as mentioned by Stevenson (1986) and Saraptaka and Krskova (1997). The observations suggest that the microbial populations might have been developed resistance towards the high metal concentrations and the phosphorus that is present in soil might not be available for the microorganisms.

Statistical analysis using Linear Pearson's Correlation and ANOVA has been performed. Phosphorus concentration (P_T, P_O and Pi) and acid phosphatase activity shows strong positive correlation (P_T- 0.86, P_O- 0.84 and Pi- 0.83) with *p* values <0.05 for both P_T and P_i but not with P_O. Alkaline phosphatase shows a significant negative correlation with soil phosphorus concentrations (r = -55) and it correlates with the data reported by Wright and Reddy (2001). The negative correlation between alkaline phosphatase and phosphorus may be due to higher labile phosphorus content of soil as reported by Puglisi et al. (2006) and Gianfreda et al. (2005).

Heavy metal analysis

From the data obtained for heavy metal concentration (Table 2), it is clear that the distribution of heavy metals is always higher in Vellore City or VIT University except Nickel, which was highest in Amirthi forest; Zinc, which was highest in Bramhapuram, and Chromium, which was highest in Ranipet.

Table 2. Heavy metal concentrations.

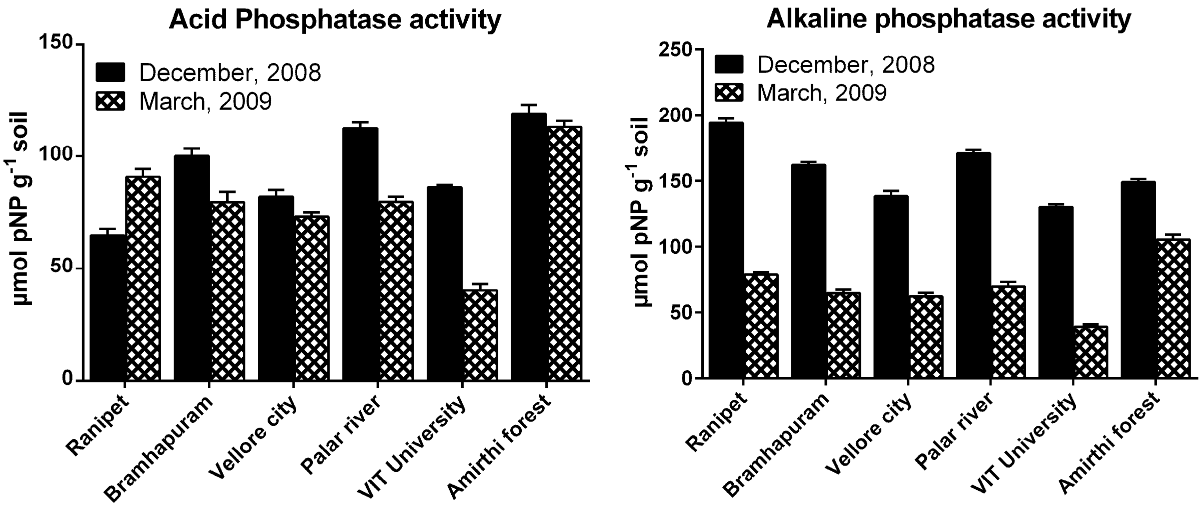
Parameters	Ranipet	Bramhapuram	Vellore City	VIT University	Palar Basin	Amirthi Forest
Iron	663.13	791.75	2735.13	2530.88	801.37	284.81
Magnesium	306.38	308.92	596.21	740.25	194.98	193.19
Cobalt	3.5	1.62	3.5	3.54	1.88	0.52
Manganese	5.63	11.37	36.37	37.25	9.38	1.76
Copper	22.25	35.01	45.53	46.57	40.75	7.62
Nickel	1.38	1.35	3.01	3.37	BDL	3.41
Lead	41.25	42.5	61.25	51.25	52.5	41.25
Chromium	151.87	53.55	55.00	55.02	33.00	35.00
Zinc	83.32	87.91	64.62	71.27	29.86	79.41

*All values are expressed in mg metal Kg^{-1} Soil

The high metal accumulation in VIT University may be due to the usage of partially treated water in which ozone is used to render soluble form of metals into insoluble form according to UNIDO (2005) or this may be due to cumulative discharge of pipe materials (Pb, Zn, and Fe) of water treatment plant according to World Health Organisation (WHO), Geneva, 2004. As compared to the other areas, Amirthi forest shows lowest metal concentrations except Nickel and Palar Basin shows nickel below detection limit (BDL). The concentration of metals was higher than those reported by Ekenler and Tabatabai (2002) in USA, and the observed values were lower than those reported by Zhang and Min (2008) in China for iron (Fe).

Bramhapuram has the high concentrations of Cu, Pb and Zn, may be due to Zinc Sulphate Heptahydrate and Copper salts which are generally present in the fertilizers. The lead (Pb) concentration is observed to be high with 42.5 mg Kg^{-1} in this site. But, Pb content is highest in Vellore city followed by Palar river and VIT University as discussed earlier.

Fig. 1: Phosphatase activity of the soil samples.



Palar river basin found to contain lower concentrations of metals after Amirthi forest compared to other locations except Pb, which exceeds the level of VIT University. A copper (40.75 mg Kg-1) concentration was found to be similar with Bramhapuram, VIT University and Vellore city. This may be due to the surface runoff from the nearby fields of Bramhapuram or other agricultural areas situated on the bank of river and also from Ranipet leather industries. There is a bore wells which have installed in the Palar basin which provides water to the residents of Vellore city and the water condition is depleting. This may cause concern about the concentrations heavy metals in the water table in the Palar basin and adverse effect on human health.

Chromium reaches highest mean concentration levels of 215.49 mg/kg^{-1} in Ranipet, due to anthropogenic pollution from tanneries, which is generally linked to the use of Cr (VI) compounds in several industrial applications such as plating, metallurgy, pigments, and leather tanning remediation. Chromium (Cr) levels are lower than those reported by Singh and Philip in 2005 (9±12 mg/g) for Ranipet area in 2005. Ranipet was reported to be the 30 most polluted places in the world according to Blacksmith Institute, New York, 2007. The high concentration of nickel in Amirthi forest may be due to the trophic transfer of the said metal which was found to be similar in concentrations with Vellore city and VIT University.

Phosphatase activity and other soil parameters

Neble et al. (2007) reported that extracellular phosphatase activity and organic phosphorus mineralisation depends on the microbial biomass content in soil. Microbial biomass shown a positive correlation with acid phosphates activity (r = 0.44, $p \leq 0.05$). However, the lowest microbial biomass was seen in the Palar river basin as expected because of dried environment with majority of soil composed of sand. Next to Palar, the lowest microbial biomass (bacteria- 161×10^{-6} CFU and fungi- 48×10^{-3} CFU) was observed in VIT University. A typical feature of soils contaminated by heavy metals is the reduction of soil microbial biomass and the change in soil activity (Brookes, 1995). This might explain how high heavy metal concentrations in the campus inhibiting the growth of microorganisms in artificially managed environment. As fungal biomass is also very low, the level of phosphate solubilisation is also low, leading to accumulation of phosphate.

It is followed by Ranipet contaminated site, where microbial biomass is lower due to high metal pollution. However, in Ranipet, the soil organic mass is high (8.8 %). It is now clear that even though, inorganic phosphorus content in Ranipet is high, acid phosphatase activity was observed to be low due to low levels of microbial biomass which is the source of enzyme other than plants. Our analyses indicate the relative heavy metal distribution and soil quality in various significant locations of Vellore. By considering that a small number of variables should be examined in developing an index of soil quality (Doran and Parkin, 1994). This study strongly proves that the high concentration of heavy metal content in soil makes nutrients in the soil to be unavailable to the plants and microorganisms and thereby decreasing biomass and biological activity. This study also confirms that the strong relationship between P availability and enzyme activity of soil and correlates with Sinsabaugh et al. (2008).

Conclusion

In this study, we have shown that the areas near Vellore are highly contaminated with heavy metals which are evidenced by the poor growth of the soil microbiota. The phosphorus is essential for normal biological processes which is made unutilized due to these heavy metals in the soil. There is a need for immediate soil rehabilitation which is necessary for normal growth of the microorganisms.

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