Assessment of heavy metal pollution and its impacts on soil physical, chemical properties and β-glucosidase activities in agricultural lands, Puducherry region

Project report submitted to

Department of Science, Technology and Environment

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Symbol	Abbreviation	Symbol	Abbreviation
CFU	colony forming unit	**Ba**	Barium
EC	Electrical conductivity	**Sr**	Strontium
N	Nitrogen	**V**	Vanadium
P	Phosphorus	**Cr**	Chromium
K	Potassium	**Cu**	Copper
Ca	Calcium	**Pb**	Lead
Na	Sodium	**Co**	Cobalt
NH_4	Ammonia	**Zn**	Zinc
NO_3	Nitrate	**mg**	Milligram
SO_4	Sulfate	**kg**	Kilogram
S	Sulphur	**µg**	Microgram
Ni	Nickel	**ppm**	Parts per million
As	Arsenic	**mS**	Milli simons
Mn	Manganese	**p-NP**	Para nitro phenol

1. INTRODUCTION

The concentrations of heavy metals in soils are associated with biological and geochemical cycles. They are influenced by anthropogenic activities, such as transport, waste disposal, industrialization, social and agricultural activities have an effect on environmental pollution and the global ecosystem. These functions lead to a negative effect on human health and on all living organisms. Pollution of the environment with toxic metals has increased suddenly since the onset of the industrial revolution. Soil pollution by heavy metals, such as cadmium, lead, chromium and copper etc. is a problem of concern (Fytianos 2001). Heavy metals are naturally present in soil eventhough heavy metal contamination comes from local sources: mostly industry (mainly non-ferrous industries, but also power plants and iron, steel and chemical industries), agriculture (irrigation with polluted waters, sewage sludge and fertilizer, especially phosphates, contaminated manure and pesticide containing heavy metals), waste incineration, combustion of fossil fuels and road traffic. Long-range transport of atmospheric pollutants adds to the metals in the natural environment. Heavy metals can be found generally at trace levels in soil and vegetation, and living organisms feel the need for micro-elements of these metals. However, these heavy metals have a toxic effect on organisms at high content levels.

1.1 Contamination through fertilizers/pesticides

Additional use of fertilizers and pesticides in agricultural activities to increase productivity due to the rapid population increase and development of technology threatens the groundwater and surface water on a large scale. In most of the countries, soils and waters have been contaminated by fertilizers and pesticides used during agricultural activities. These waters and territories continue to be polluted, as the necessary precautions have not been called for. This indicates there is an obvious risk for human in the future (Smith *et al.,* 1971). Organic materials such as farm manures, bio-solids or composts contain higher concentration of trace elements than most agricultural soils. The use of bio-solids and compost increases the total amount of Cu, Zn, Pb, Cd, Fe and Mn in soils (Tulay Ekemen Keskin 2010).

The use of phosphate fertilizers in agricultural field has shown to enhance leaching of Cd from soil, which reaches the lake water. It undergoes physical and chemical changes depending on the pH and quality of water and sediment. The available metals in the water phase cause a danger to human beings and biota. Carbon and Nitrogen concentration increase in response to irrigation, but it is not clear whether this is due to decreased decomposition rate of crop residues in response to pollution in the irrigation water or to increased amounts of crop residue in the irrigated soils (McClean 2003).

1.2 Effects of heavy metals

Heavy metal contaminated soil adversely affects the whole ecosystem when these toxic heavy metals migrate into groundwater or are taken up by flora and fauna, which may result in great threat to ecosystems due to translocation and bioaccumulation. Heavy metals are potentially toxic to crop plants, animals, and human beings when the contaminated soils are used for crop production. Environmental pollution of the biosphere with heavy metals due to intensive agricultural and other anthropogenic activities poses serious problems for secure usage of farming land (Wong *et al.,* 2002).

Intake of vegetables is an important path of heavy metal toxicity to human beings. Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals, it depends upon the nature of vegetables and some of them have a greater potential to accumulate higher concentration of heavy metals than others. Dietary intake of heavy metals through contaminated vegetables may contribute to several chronic diseases. The sources of heavy metals to vegetable crops are growth media (soil, air, nutrient solutions) from which they are taken up by the roots or foliage (OdohRapheal and Kolawole Sunday Adebayo 2011).

Heavy metal toxicity has an inhibitory effect on plant growth, enzymatic activity, stoma function, photosynthesis activity and accumulation of other nutrient elements and also damages the root system. To the concern of the soil however, the effects of heavy metals pollutants could be enormous. Major amongst which is their effects on microbial activities (Wyszkowska, 2002). Other negative effects of heavy metals, especially as they are being discharged through industrial

effluents include negative effects on porosity and water holding capacity, CEC, mineral composition and seed germination. All heavy metals are toxic at soil concentrations above normal level (Ayolagha and Nleremchi, 2000). The CEC of the soil is a key factor in determining heavy metal concentration and even availability in the soil. As CEC is determined by organic matter content and clay type and quantity, one is invariably saying that organic matter content and clay content affect concentration of heavy metals in the soils.

1.3 Soil enzymes

An enzyme is a substance, composed of protein that is capable of lowering the activation energy of other selective compounds enough to allow the breaking of a particular bond under a particular environment. So, such reactions influenced by enzymes are called biological reactions. The action of enzymes to make a split easier does not "use up" the enzyme. Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Burns, 1983; Sinsabaughb *et al.,* 1991).

1.4 β-Glucosidase

β-glucosidase is a common and predominant enzyme in soils (Eivazi and Tabatabai, 1988). It is named according to the type of bond that it hydrolyses. This enzyme plays an important role in soils because it is involved in catalysing the hydrolysis and biodegradation of various β-glucosides present in plant debris decomposing in the ecosystem. Its final product is glucose, an important Carbon energy source of life to microbes in the soil. Several researchers have however also reported its phytopathological effects in the ecosystem For example, some of the a glycons are known to be the precursors of the toxic substances which cause soil sickness where plants are grown as monocrops. β-glucosidase enzyme is very sensitive to changes in pH, and soil management practices. Acosta-Martinez and Tabatabai (2000) reported β-glucosidase as sensitive to pH changes. This property can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification in situations involving activities of this enzyme. β-glucosidase enzyme is also known to be inhibited by heavy metal contamination such as Cu and several others. For instance, studies have shown that plant debris did not decompose or show β-

glucosidase activities when exposed to heavy metal polluted soils (Geiger *et al.,* 1993). Consequently, more understanding of the β-glycosidase enzyme activities and factors influencing them in the ecosystem may contribute significantly to soil health studies.

1.5 Agriculture in India

Agriculture is demographically the broadest economic sector which plays a significant role in the overall socio-economic fabric of India. In India, the majority of farmers hold less than 2 hectares of land. The Indian coastal region has long been agriculturally productive, especially for intensive rice cultivation with good irrigation support. Most of the agricultural soils of India are characterized by arable, semi-arid, low in soil organic carbon (SOC) and macro and micronutrients. The agricultural system in India is typically a monsoon-driven low-input farming with limited use of organic amendments. The inorganic chemical fertilizers with inadequate organic amendments are used primarily to meet the gap between the soil reserve and crop requirement. However, such farming practices affects the physical, chemical, mineral, soil biological processes and biochemical properties of soil.

1.6 Agriculture in Puducherry

Out of 20 distinct agro ecological region identified by the Indian Council for Agricultural Research in India, the coastal agro ecosystem is one among them and with its own peninsular physical/ecological features. The Pondicherry region located in the east coast has a coastal length of 22 km with narrow coastal lines. Out of major 3 land forms namely marine, fluvial and uplands, the fluvial plains are extensively cultivated while the other forms are marginally used for agriculture.

A wide range of crop exhibiting rich crop diversity in Pondicherry, food crops are cultivated in 82.93% of the total cultivated area. Paddy is the principle crop and mostly 3 cropping seasons of paddy are being cultivated in a year. Besides paddy, the following crops like ground nut, black gram, green gram and bajra such as banana and sugarcane are cultivated in mono cropping system. However on perusal of yield gap in paddy with different soil series of Puducherry region for paddy

shows a definite gap of 8% to 21 % in production of per hectare. This gap due to improper soil management practices, imbalance usage of fertilizer, salinity and water logging. In Puducherry region most of the farmers are follow mono cropping system which leads to leaching of soil nutrients, surveillance of highest disease and pest attack and reduction of crop yield. Indiscriminate usage of inorganic fertilizer and synthetic pesticides enhance ecological imbalance, soil salinity and health hazards.

2. OBJECTIVES

1. Analysis of physical, chemical and biological properties of soil in agriculture lands of Puducherry region.

2. To evaluate the presence of toxic heavy metals (Cu, Zn, Pb, Cd, Hg , Cr and Mg) in agriculture lands of the region.

3. To assess the relationship between soil chemical and biological factors.

4. Assessing impacts of heavy metal pollution on soil physical, chemical properties and β-glucosidase activities.

3. LITERATURE REVIEW

Walker (1954) reported that, soils weathered from ultramafic rock, often also referred to as ultrabasic or serpentine soils, pose special challenges for plant growth and survival. These rocks and their resulting soils are characterized by high levels of metals (e.g. nickel, cobalt); low levels of nitrogen, phosphorus, and potassium; high levels of magnesium with low calcium; and low soil moisture**.** Ultrabasic outcrops often have poor productivity and contain many endemic species that are specially adapted to the potentially toxic levels of magnesium and other metals.

Singh *et al*., (1989) have also reported a seasonal variation in the microbial C, N and P in forest and savanna. Higher microbial growth utilizes phosphorous, potassium and magnesium and causes mineralization of nitrogen hence, amount of phosphorous, potassium and magnesium decreased and of available nitrogen increased during monsoon compared to pre monsoon season. Previous interpretation of soil CO_2 fluxes emphasized particularly on the measurement of total, which was usually separated into two components: root respiration and microbial respiration. However, rhizomicrobial respiration was not distinguished from root-free soil microbial respiration.

Brookes (1995) says that the microbial parameters appear very useful in monitoring soil pollution by heavy metals, but no single microbial parameter can be used universally. Microbial activities such as respiration, C and N mineralization, biological N_2 fixation and some soil enzymes can be measured. Combining microbial activity and population measurements (e.g., biomass specific respiration) appears to provide more sensitive indications of soil pollution by heavy metals than either activity or population measurements alone. He concluded that the fertility of natural ecosystems, however, depends almost entirely on natural microbial processes, including N_2 fixation, the mineralization of organic forms of N, C, P and S and organic matter transformations, all mediated by the soil microbial biomass. Any decline in natural fertility resulting from pollutants entering soils will therefore have proportionately greater effects on natural ecosystems.

Barbara Wick *et al.,* (1998) identified, soil microbiological and soil biochemical parameters (pH, exchangeable basic cations, inorganic and organic phosphorus pools, total organic carbon and total nitrogen, microbial biomass carbon, acid and alkaline phosophatase, β-glucosidase and protease activity) as indicators of soil quality under improved fallow management systems with *Senna, Leucaena and Pueraria* on severely degraded and non-degraded soil. They report that, *Pueraria* sustained soil quality on the non-degraded site but did not improve the severely degraded site, suggesting that *Pueraria* is a soil fertility maintenance crop. In contrast, S*enna* improved the degraded sites and more soon the most severely degraded site. Apparently, *Senna* can be considered as a suitable plant for soil restoration purposes.

Simek *et al.,* (1999) using soils from field plots in four different arable crop experiments that have received combinations of manure, lime and inorganic N, P and K for up to 20 years, the effects of these fertilizers on soil chemical properties and estimates of soil microbial community size and activity were studied. The soil pH was increased or unaffected by the addition of organic manure plus inorganic fertilizers applied in conjunction with lime, but decreased in the absence of liming. The soil C and N contents were greater for all fertilized treatments compared to the control, yet in all cases the soil samples from fertilized plots had smaller C: N ratios than soil from the unfertilized plots. He found that the result indicates the difference in the composition or function of microbial communities in the soils in response to long-term organic and inorganic fertilization, especially when the soil was not limited.

Zhangrennan *et al.*, (1999) studies details with relations between soil properties and selected heavy metal concentrations in spring wheat (*Triticum aestivum* L.) grown in contaminated soils. The soil samples were analyzed for pH, organic matter and available phosphorous (P); also for total cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) contents.

Aydinalp (2003) determined the levels of the heavy metals, cadmium (Cd), copper (Cu), lead (Pb), manganese (Mn), nickel (Ni) and zinc (Zn) in the agricultural soils of the Bursa plain so that the degree of pollution could be ascertained. The study also identified the various heavy metal forms present in soils using a fractionation scheme based on sequential extraction.

Krishna and Govil (2007) studied about soil contamination due to heavy metals from an industrial area of Surat, Gujarat, and Western India. The study was undertaken on soil contamination in Surat, Gujarat (India). They determined the extent and distribution of heavy metals like Ba, Cu, Cr, Co, Ni, Sr, V and Zn.

Weixin Ding *et al.,* (2007) evaluated the response of soil respiration to soil moisture, temperature, and N fertilization, and estimate the contribution of soil and rhizosphere respiration to total soil CO_2 emissions. A seasonal soil CO_2 emission in the CK, N0, N150, and N250 treatments was estimated to be 294, 598, 541, and 539 g Cm^{-2} respectively. The seasonal soil CO_2 fluxes were significantly affected by soil temperature, with the change in the rate of flux for each 10°C increase in temperature ($Q10$) of 1.90 to 2.88, but not by soil moisture. Nitrogen fertilization resulted in a 10.5% reduction in soil CO_2 flux; however, it did not significantly increase the maize aboveground biomass but did increase maize yield. Soil respiration measurement using the root-exclusion technique indicated that soils fertilized with 150 kg N ha−1contributed 54%of the total soil CO_2 emission, or 8% of soil organic C down to a depth of 40 cm. An amount of C equivalent to 26% of the net assimilated C in harvested above and below ground plant biomass was returned to the atmosphere by rhizosphere respiration.

Ademir *et al.,* (2009) studied soil under organic agricultural system presents higher microbial activity and biomass and lower bulk density than the conventional agricultural system. They showed minor differences among the selected variants in the reactive and basal respiration activity. Statistically significant differences among the variants with different fertilization were found mainly in the potential respiration activity. The ratio between the values of the basal respiration activity indicates the stability of the soil organic matter. According to this criterion, stability of the

soil organic matter was higher in the fields cropped in a nine-year crop rotation than in the field B alternatively cropped with spring wheat and sugar beet. Organic and mainly mineral fertilization increased the stability of the soil organic matter.

Dasaram *et al.,* (2010) assessed the soil contamination in Patancheru Industrial Area, Hyderabad, Andhra Pradesh, India was carried out. It involved the study of toxic metals such as Cr, Cu, Ni, Pb, Zn, including Ba, Co and V in representative soil samples from Patancheru industrial area near Hyderabad, Andhra Pradesh. Toxic trace metal geochemical studies were carried out in fifteen representative soil samples collected from residential and agricultural area, to understand the spatial distribution and to assess the level of contamination on the basis of index of geoaccummulation, enrichment factor, contamination factor and degree of contamination.

Flores-Magdaleno *et al.,* (2011) investigated the concentration of heavy metals in agricultural soils and waste water used for irrigation in plots of Mixquiahuala, Hidalgo. It analyzed the potential of hydrogen (pH), electrical conductivity (EC) and total extractable heavy metals in water and soil: As, Cd, Cr, Hg, Ni, Pb and Zn. Heavy metals were determined by using an Inductively Coupled Plasma (ICP) Perkin Elmer Optima 5300 (Inductively Coupled Plasma), using the methods recommended by the EPA (Environmental Protection Agency) and APHA (American Public Health Association).

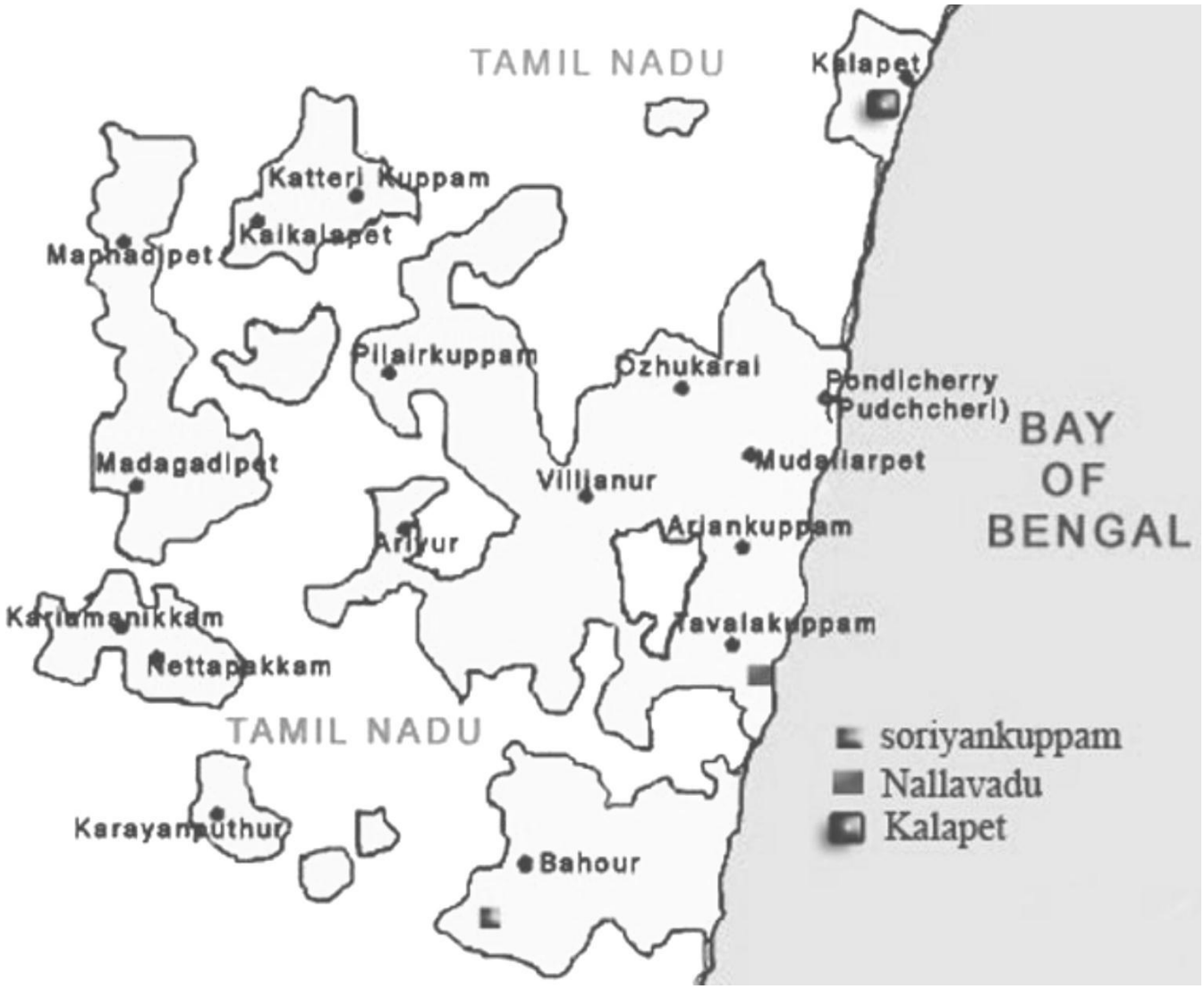
4. MATERIALS AND METHODS

4.1 study area

Puducherry is located along the Coromandel coast of peninsular India with the geographical coordinates $11^0$52'N, $79^0$45'E and $11^0$59'N and $79^0$52' E covering an area of 480 km. The mean annual rainfall of the study area is about 1311-1172 mm. The mean number of annual rainy days is 55; the mean monthly temperature ranges between 21^0 C and 30^0 C in the study area. This region gets more rainfall during north east monsoon. Humidity is also high in this region as the study area is located near the coast. The study sites, Soriyankuppam, Bahour and Kuruvinatham is located 25 km away from the Puducherry city towards to Cuddalore district, Nallavadu site is 10 km away from Puducherry city and the other site is Kalapet, located 11 km from the city. Rice, Groundnut and Sugarcane are the predominant crops cultivated in the study area. During the study period groundnut was the predominant crop grown in the sample farms**.**

Soil from 10 agricultural farms were sampled from June 2013 to December 2014. They were located in Kalapet (farm 1& 2), Kuruvinatham (farm 3&4), Soriyankuppam (farm 5&6), Bahour (7&8) and Nallavadu (farm 9&10). Three composite soil samples were collected from each of the 10 farms. Composite samples were done by sampling approximately 15 kg of soil from each of the three farming system using augur at 0-15cm depth. Bulked samples were kept separately according to the location within each field for replication maintenance. Composite soil samples were stored in deep freezer to control microbial and enzyme activities for soil dilution, plating and biological analysis. The soil was transferred to the storage room and was stored at 40^0c until the time of analysis. Microbial and enzyme analysis were done within 48 to 72 hrs.

Figure : 1 Study area map



4.2 Methodology

Table1: **Details of the analytical methods**

Physical Properties	**Analytical method**	**Reference**
Soil bulk density	Volumetric flask method	Bashour and sayegh 2007
Volume of soil particle	Volumetric flask method	Bashour and sayegh 2007
Particle density	Volumetric flask method	Bashour and sayegh 2007
Water holding capacity	Gravimetric method	Rosa Margesin and Franz Schinner 2005
Physico-chemical properties		
Soil reaction (pH) (1:2 soil water suspension)	Potentiometry	Jackson 1973
Soil salinity(E C) (1:2 soil water suspension)	Conductometry	Jackson 1973

Chemical properties		
Total Nitrogen	Macro-Kjeldahl digestion	Piper 1966
Total Phosphorus	WD-XRF	Becckhoff *et al*., 2006
Total Potassium	WD-XRF	Becckhoff *et al*., 2006
NH_4^+ - Nitrogen	Nitroprusside catalyst method	Bashour and sayegh 2007
NO_3 - Nitrogen	Chromotrophic acid spectrophotometric method	Sims and Jakson 1971
Extractable Phosphorus	0.5 M $NaHCO_3$	Olsen *et al*., 1954
Exchangeable Potassium (K^+)	Neutral normal NH_4OAc (Flame photometry)	Stanford and English 1949
SO_4 – Sulphur	Turbidimetric method	Tendon 1991
Exchangeable Sodium (Na^+)	Neutral normal NH_4OAc (Flame photometry)	Alban and Mildred Kellogg 1959
Exchangeable Calcium	Neutral normal NH_4OAc (Flame photometry)	Alban and Mildred Kellogg 1959

Biological properties		
Soil respiration	Closed jar method	Isermeyer 1952
β – glucosidase	Determination of para nitrophenol release after the incubation of soil with para nitrophenylglucoside solution for 1 h at 37^0C	Tabatabai 1982; Eivazi and Tabatabai 1988
Microbial population	Serial dilution plate count method	Germida 1993
Heavy metals		
Ba, Sr, V, Cr, Ni, Pb, Cu, Zn, As, Mn and Co	WD-XRF	Becckhoff *et al*., 2006

Wave Length Dispersive X-Ray Fluorescence Spectrometer (WD XRF)

Make: Bruker , Model : S4 PIONEER

Principle:

X-ray fluorescence analysis is a fast, non-destructive and environmentally friendly analysis method with very high accuracy and reproducibility. All elements of the periodic table from Beryllium to Uranium can be measured qualitatively, semi quantitatively and quantitatively in powders, solids and liquids. Rhodium is used as the standard anode material. The tube and generator are designed for a permanent output of 4 kW. The detector is scintillation counter and proportional counter. Besides the standard collimators with aperture angles of 0.15° and 0.46° two additional collimators can be installed to optimize the measurement parameters, depending on the application. A 0.077° collimator is available for high resolution measurements (e.g. with LiF (420). Collimators with a low resolution (e.g.1.5 – 2.0°) are advantageous for light elements such as Be, B and C as the OVO-Multilayer's angle resolution is limited.

4.3 Enumeration of soil Microorganisms by serial dilution plate technique

Materials required

1. Soil samples
2. Sterile water blanks
3. Sterile pipettes
4. Sterile petri dishes
5. Sterile media-Nutrient agar medium or soil Extract agar medium, Matrin's Rose Bengal agar medium, Kenknights agar or Kuster's agar medium.

6. Streptomycin solution(30 mg/ml)

Procedure

- Weight 10 g of the representative soil sample and transfer to 100 ml sterile water blanks contained in the 250 ml Erlenmeyer flask and shake well in a rotary shaker for 5-10 minutes(10~1 dilution or 1/10 dilution)

- preparation dilution of the suspension through 10^{-2} to 10^{-6} using 90 ml sterile water blank by transferring 10 ml of the dilution respectively

- Pipette out 1 ml form 10^{-4} dilution into 3 petridishes for fungi soil sample, 10^{-5} dilution into 3 petridishes for the soil sample of actinomycetes and 10^{-7} dilution into 3 petridishes for the soil samples of bacteria aseptically in the laminar flow chamber.

- Melt the respective agar media and cool them down to 42-45 ^{O} C (Agar media are melted well ahead cooled and held in water bath maintained at 45-48 ^{O} C). Add 10-15 ml of the nutrient agar media with the respective dilution for bacteria; add the Martin's rose Bengal agar media for fungi and the kenknight's agar media for the actinomycetes respectively.

- The diluents and the agar are mixed carefully by rotating the petridishes both in clockwise and in anti-clockwise directions.

- When the agar sets invent the petridishes and incubate at 30^{O} C.

Observations

1. Count the number of colonies of bacteria from 2-5 days, actinomycetes form 7-9 days and fungi from 3-5 days.

2. Determine the moisture percentage of the soil to express the results on over dry basis.

3. Calculate the average count/plate and express the microbial population percent of oven dry soil using the following formula.

$$\text{Microbial Population} = \frac{\text{Average number of colonies}}{\text{Dry weight of soil taken on over dry basis}} \times \text{dilution factor}$$

UNIT:

For bacteria…… ……CFU/g × 10^7 of oven dry soil

For fungi……. CFU/g × 10^4 of oven dry soil

For actinomycetes……... CFU/g × 10^5 of oven dry soil

(CFU-colony forming unit)

Table 2: Medium used for microbial analysis

Medium	**Microbes**
Nutrient Agar	Bacteria
Rose Bengal	Fungi
Ken knight's	Actinomycetes

4.4 Data analysis

All the experimental data were analyzed with SPSS/16. The relationship among heavy metals, soil chemical and biological properties were analyzed by person correlation.

5. RESULTS AND DISCUSSION

Table 3: Heavy metals concentration in soils from selected agriculture farms

Sample	farm 1	farm 2	farm 3	farm 4	farm 5	farm 6	farm 7	farm 8	farm 9	farm 10	MPC
Cr (PPM)	58 ±4	51 ±5	66 ±9	42 ±6	48 ±4	47 ±7	27 ±2	45±3	48±3	39±4	100
Ni (PPM)	13±1	10±1	21±2	13±1	17±3	19±2	9±1	13±2	15±1	12±1	80
Cu (PPM)	6±1	8±2	16±3	12±2	14±1	16±4	7±1	11±1	10±1	12±2	30
Zn (PPM)	36±3	53±2	42±3	29±4	43±1	31±5	19±2	39±4	31±2	36±2	200
As (PPM)	5±1	6±1	6±2	5±2	5±1	6±1	4±1	5±1	2±1	7±1	12
Pb (PPM)	7±2	10±3	12±2	11±1	10±2	10±1	7±2	15±2	16±2	13±3	70
Mn (PPM)	52±5	58±2	87±6	134±9	66±4	155±6	112±4	106±4	102±2	62±3	
V (PPM)	40±2	36±1	58±1	35±1	47±3	51±2	27±1	33±3	39±2	36±3	100
Co (PPM)	6±1	5±1	8±1	6±2	8±1	9±2	5±1	6±1	5±1	5±2	17
Sr (PPM)	24±2	32±1	331±6	172±9	180±6	140±5	82±2	156±3	336±11	196±6	200
Ba (PPM)	78±5	113±3	627±15	396±10	428±12	431±3	298±8	366±12	654±13	395±19	300

±: Standard error and MPC: Maximum permissible concentration in soil by WHO, 1996

Table 4: Soil physical and physico chemical properties of selected agriculture farms

Soil physical parameters	farm 1	farm 2	farm 3	farm 4	farm 5	farm 6	farm 7	farm 8	farm 9	farm 10
Soil bulk density g/cm^3	1.18 ±0.1	1.18 ±0.3	1.25 ±0.2	1.11 ±0.4	1.05 ±0.2	1.00 ±0.3	1.18 ±0.1	1.25 ±0.5	1.11 ±0.2	1.05 ±0.4
Volume of soil particle cm^3	23.4 ±1.6	27.3 ±1.8	25.4 ±1.3	23.0 ±1.6	25.8 ±1.2	24.4 ±1.5	24.5 ±0.9	24.4 ±1.8	24.2 ±1.4	23.9 ±1.6
Particle density g/cm^3	2.1 ±0.1	1.8 ±0.2	2.0 ±0.1	2.2 ±0.3	1.9 ±0.2	2.0 ±0.4	2.0 ±0.1	2.0 ±0.4	2.1 ±0.2	2.1 ±0.3
Water holding capacity %	62.3 ±2.5	64.2 ±1.6	80.4 ±3.2	73.2 ±2.9	70.1 ±3.5	80.1 ±4.2	76.5 ±2.5	80.1 ±3.1	78.0 ±2.4	70.6 ±2.4
pH	6.31 ±0.82	7.56 ±0.79	7.2 ±0.93	6.61 ±1.02	7.52 ±0.82	7.84 ±0.96	7.92 ±0.53	8 ±1.03	7.04 ±0.95	6 ±1.10
EC (mS/cm)	0.069 ±0.022	0.074 ±0.015	0.298 ±0.023	0.258 ±0.012	0.43 ±0.052	0.242 ±0.032	0.135 ±0.025	0.353 ±0.091	0.232 ±0.034	0.388 ±0.071

±: Standard error

Table 5: Soil chemical properties of selected agriculture farms

Soil chemical parameter	farm 1	farm 2	farm 3	farm 4	farm 5	farm 6	farm 7	farm 8	farm 9	farm 10
Total nitrogen (g/kg)	2.55 ±0.12	2.05 ±0.15	3.57 ±0.25	1.78 ±0.14	1.85 ±0.10	4.89 ±0.52	1.59 ±0.24	1.99 ±0.16	2.6 ±0.36	2.36 ±0.41
Total phosphorus(g/kg)	1.12 ±0.05	0.92 ±0.08	2.38 ±0.09	1.73 ±0.10	1.7 ±0.11	2.34 ±0.13	1.48 ±0.05	1.9 ±0.09	1.4 ±0.07	1.2 ±0.04
Total potassium (g/kg)	15.6 ±1.23	17.5 ±1.09	50.97 ±0.98	37.9 ±1.35	33.2 ±1.69	44.2 ±2.01	28.2 ±1.89	41.8 ±2.39	62.2 ±2.93	35.4 ±1.24
Organic carbon (g/kg)	6.15 ±1.25	6.05 ±0.96	8.5 ±1.35	6.9 ±0.85	7 ±1.42	9.6 ±1.91	7.05 ±1.28	7.5 ±1.53	7.8 ±0.96	8.4 ±1.22
NH_4^+-N (g/kg)	0.72 ±0.01	1 ±0.02	1 ±0.04	0.81 ±0.01	0.64 ±0.02	0.49 ±0.01	0.63 ±0.03	0.5 ±0.01	1.1 ±0.01	0.65 ±0.05

±: Standard error

Table 6: Soil chemical properties of selected agriculture farms

Soil chemical parameter	farm 1	farm 2	farm 3	farm 4	farm 5	farm 6	farm 7	farm 8	farm 9	farm 10
NO_3 -N (g/kg)	1.4 ±0.04	0.89 ±0.01	2.17 ±0.09	0.89 ±0.07	1.23 ±0.05	2.5 ±0.08	0.81 ±0.01	1.09 ±0.05	2.1 ±0.09	1.03 ±0.01
Extractable Phosphorus (g/kg)	0.41 ±0.002	0.25 ± 0.001	0.61 ± 0.003	0.56 ± 0.006	0.19 ± 0.007	0.59 ± 0.004	0.35 ± 0.002	0.27 ± 0.006	0.62 ± 0.003	0.27 ± 0.004
Extractable Potassium (g/kg)	0.23 ±0.004	0.23 ±0.007	0.28 ± 0.002	0.27 ±0.008	0.24 ±0.002	0.29 ±0.005	0.26 ±0.003	0.26 ±0.009	0.36 ±0.006	0.27 ±0.008
SO_4- Sulphate (g/kg)	0.51 ±0.001	0.54 ±0.002	1.14 ±0.005	0.57 ±0.001	0.57 ±0.002	0.83 ±0.003	0.5 ±0.006	0.82 ±0.005	0.96 ±0.002	0.86 ±0.003
Extractable Calcium(mg/Kg)	15.03 ±1.02	2.04 ±0.65	5.01 ±0.96	12.02 ±1.10	10.02 ±0.93	12.02 ±1.14	9.018 ±0.85	17 ±1.87	19 ±1.56	10.4 ±1.24
Extractable Sodium (mg/Kg)	536 ±10	538 ±20	490 ±12	502 ±19	506 ±36	478 ±28	518 ±32	510 ±24	752 ±41	506 ±38

±: Standard error

Figure: 2

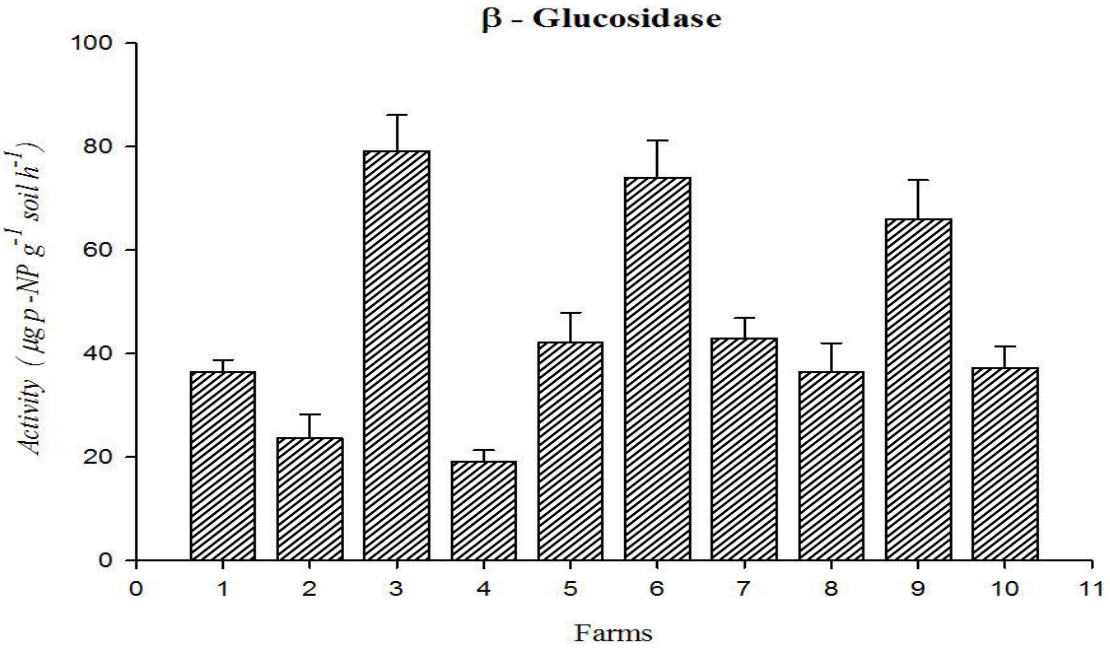


Figure: 3

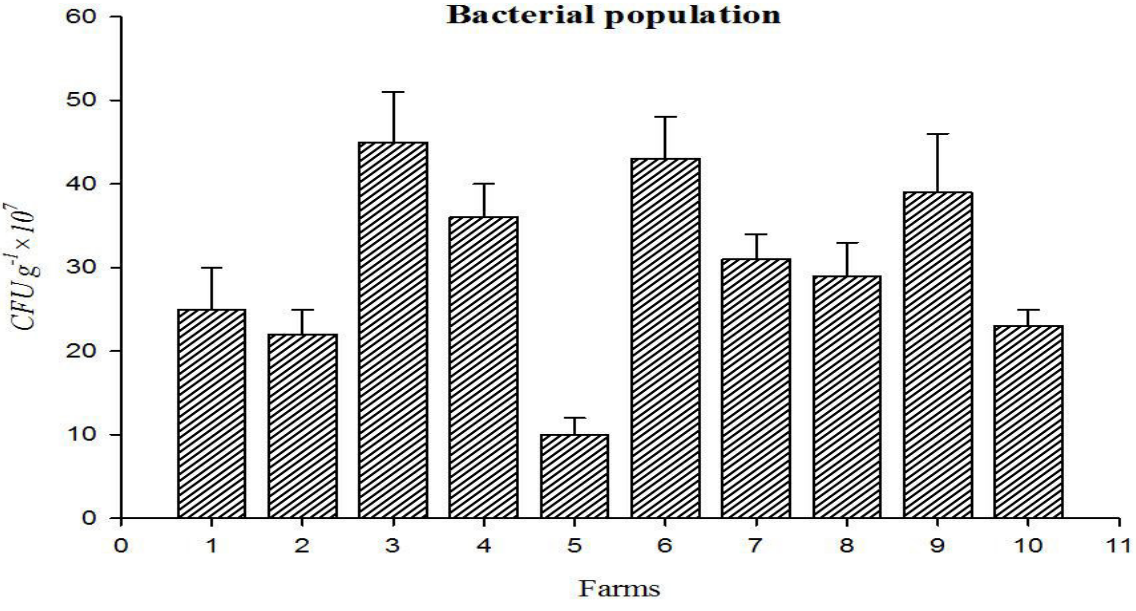


Figure: 4

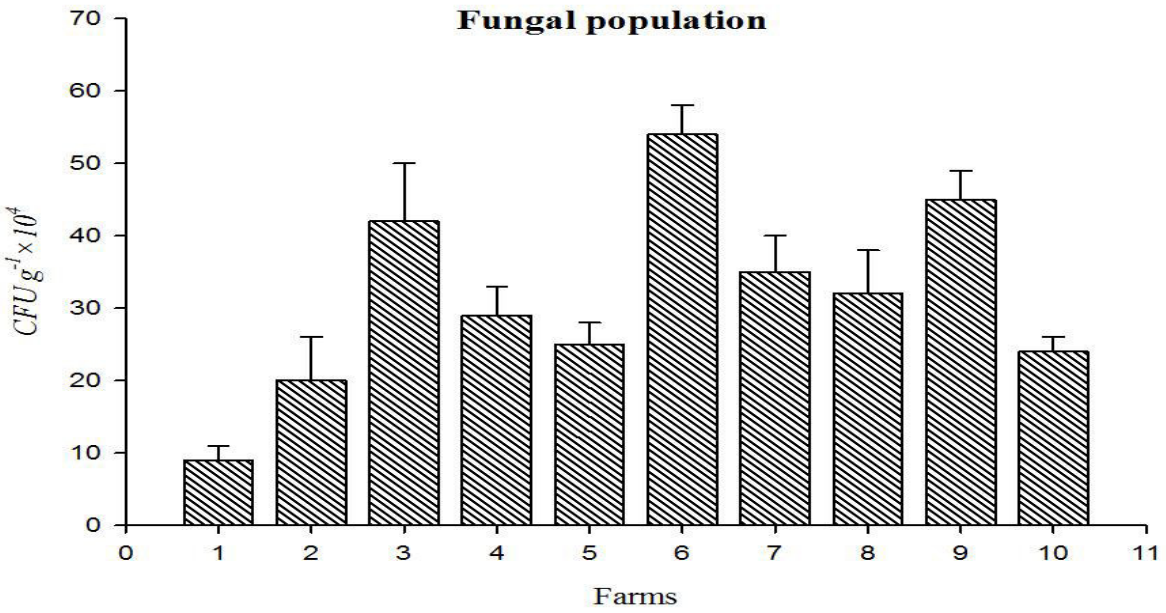


Figure: 5

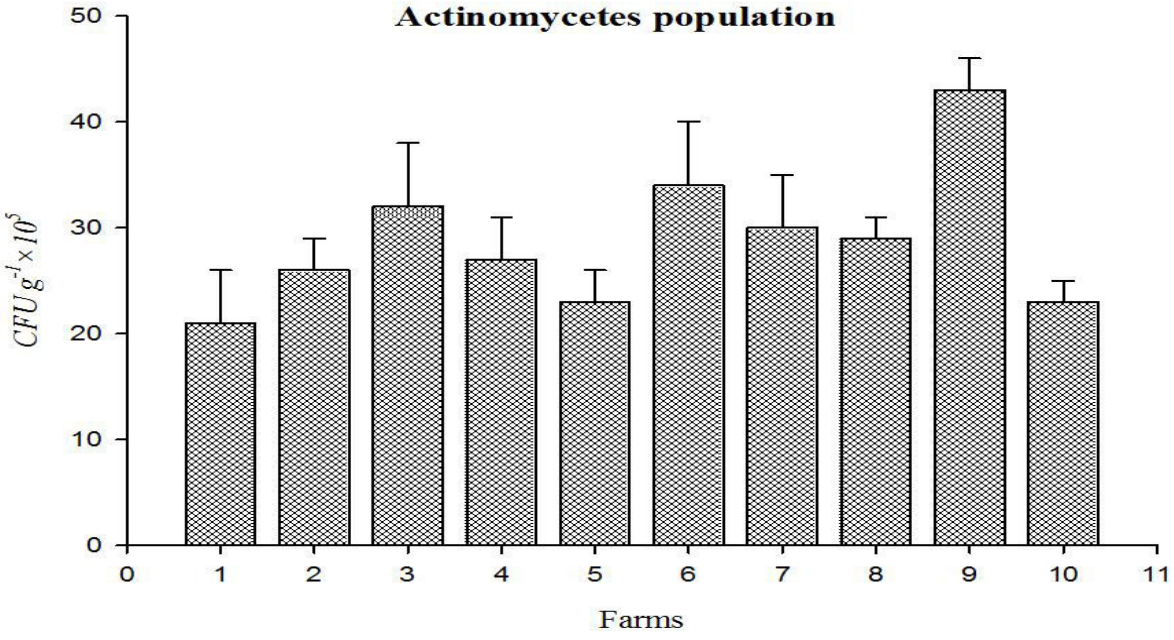


Figure: 6

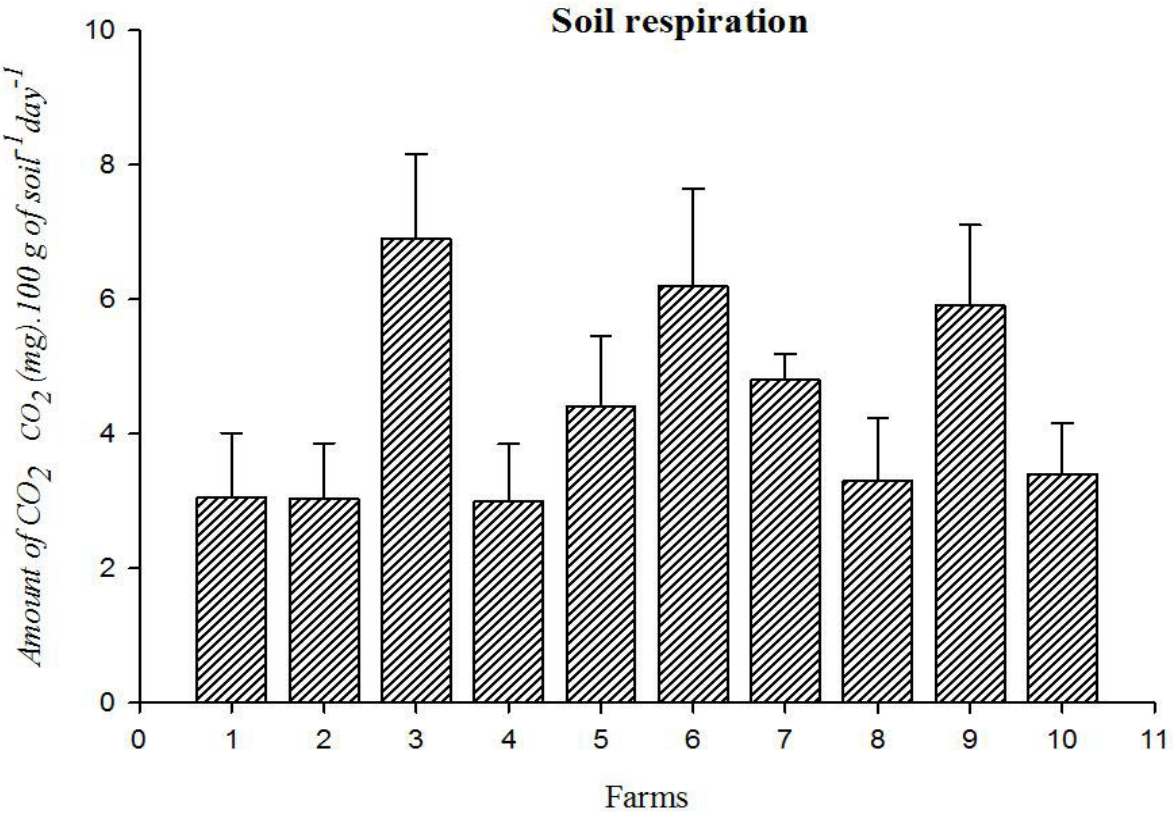

Soil respiration
Amount of CO_2 CO_2 (mg).100 g of soil^{-1} day^{-1}
0
2
4
6
8
10
0
1
2
3
4
5
6
7
8
9
10
11
Farms

Table 7: Correlation among soil biological parameter and soil chemical parameter

	β-glucosidase	Soil respiration	Bacteria	Fungi	Actinomycetes
Total nitrogen	0.779**	0.672*	0.619	0.622	0.388
Total phosphorus	0.656*	0.671*	0.595	0.731*	0.368
Total potassium	0.680*	0.697*	0.628	0.805**	0.816**
Organic carbon	0.756*	0.697*	0.585	0.803**	0.482
NH_4^+-N	0.130	0.217	0.241	Ns	0.386
NO_3 -N	0.915**	0.830**	0.648*	0.700*	0.634*
Extractable Phosphorus	0.612	0.631	0.907**	0.634*	0.682*
Extractable Potassium	0.602	0.618	0.637*	0.734*	0.903**
SO_4- Sulphate	0.754*	0.678*	0.609	0.628	0.581
Extractable Calcium	0.088	Ns	0.137	0.139	0.330
Extractable Sodium	0.187	0.191	0.144	0.164	0.649*

*correlation is significant at the ≥0.05 level of interval, Ns: Not significant

**correlation is significant at the ≥0.01 level of interval

Table 8: Correlation among heavy metals and soil physical and chemical parameters

	Cr	**Ni**	**Cu**	**Zn**	**As**	**Pb**	**Mn**	**V**	**Co**	**Sr**	**Ba**
Soil bulk density	0.298	Ns	Ns	0.198	Ns	0.041	Ns	ns	Ns	Ns	Ns
Volume of soil particle	0.250	0.043	0.074	0.714*	0.205	Ns	Ns	0.227	0.108	Ns	Ns
Particle density	Ns	0.015	Ns	Ns	Ns	0.189	0.326	Ns	Ns	0.298	0.267
Water holding capacity	Ns	0.475	0.578	Ns	Ns	0.496	0.729*	0.242	0.351	0.644**	0.774**
pH	Ns	0.085	0.136	Ns	Ns	Ns	0.416	0.014	0.302	Ns	0.068
EC	Ns	0.439	0.704*	0.085	0.201	0.542	0.076	0.282	0.361	0.576	0.605
Total nitrogen	0.446	0.732*	0.598	Ns	0.267	0.051	0.398	0.743*	0.690*	0.257	0.330
Total phosphorous	0.238	0.791*	0.816**	Ns	0.122	0.181	0.658*	0.639*	0.806**	0.490	0.619
Total potassium	0.110	0.588	0.596	Ns	Ns	0.760*	0.502	0.370	0.247	0.918**	0.956**
Organic carbon	0.028	0.643*	0.766**	Ns	0.220	0.228	0.538	0.524	0.538	0.563	0.673*
NH_4-N	0.444	0.065	Ns	0.301	Ns	0.174	Ns	0.163	Ns	0.417	0.261
NO_3-N	0.533	0.830**	0.569	Ns	Ns	0.249	0.352	0.779**	0.631	0.542	0.587

*correlation is significant at the ≥0.05 level of interval, Ns: Not significant

**correlation is significant at the ≥0.01 level of interval

Table 9: Correlation among heavy metals and soil chemical and biological parameters

	Cr	**Ni**	**Cu**	**Zn**	**As**	**Pb**	**Mn**	**V**	**Co**	**Sr**	**Ba**
Extractable Phosphorus	0.314	0.527	0.329	Ns	Ns	0.385	0.589	0.430	0.284	0.527	0.551
Extractable Potassium	Ns	0.332	0.277	Ns	Ns	0.532	0.469	0.147	Ns	0.767**	0.796**
So_4- Sulphate	0.422	0.662*	0.625	0.095	0.044	0.705*	0.174	0.569	0.288	0.840**	0.766**
Extractable Calcium	Ns	0.022	Ns	Ns	Ns	0.412	0.278	Ns	Ns	0.213	0.230
Extractable Sodium	0.019	Ns	Ns	Ns	Ns	0.404	Ns	ns	Ns	0.402	0.331
β -glucosidase	0.380	0.778**	0.563	Ns	Ns	0.228	0.416	0.014	0.571	0.626	0.687*
Soil respiration	0.270	0.732*	0.572	Ns	Ns	0.174	0.076	0.282	0.546	0.655*	0.742*
Bacterial population	0.191	0.429	0.334	Ns	Ns	0.249	0.398	0.743*	0.288	0.473	0.523
Fungal population	Ns	0.548	0.612	Ns	Ns	0.385	0.650*	0.639*	0.438	0.596	0.753*
Actinomycetes Population	0.005	0.319	0.221	Ns	Ns	0.532	0.502	0.370	0.041	0.620	0.695*

*correlation is significant at the ≥0.05 level of interval, Ns: Not significant

**correlation is significant at the ≥0.01 level of interval

6. Result and Discussion

6.1. 1Heavy metal in soil

The individual results obtained for each metal and Maximal permitted threshold soil concentrations of potentially toxic metals prescribed by WHO guidelines (WHO, 1996) were also given in table 3. Among soil in 10 farms heavy metal chromium concentration was varied between 27- 66 mg/kg, followed by Ni 9-17 mg/kg, Cu 6-16 mg/kg, Zn 9- 43 mg/kg, As 2-7 mg/kg, Pb 7 – 16 mg/kg, Mn 52 -152mg/kg, V 27-58 mg/kg, Co 5-9 mg/kg, Sr 21- 336 mg/kg and Ba 78-654 mg/kg. The ranking order of occurrence of the heavy metals in 10 farms soils was Ba>Sr>Mn>Cr>V>Zn>Ni>Cu>Pb>Co>As indicating that Ba concentration was high. Cr, Ni, Cu, and V heavy metal concentration were high in farm 3 soil. Pb, Sr and Ba concentration were high in farm 9 soil. Mn and Co concentration were higher in farm 6, followed by Zn and As concentration were high in farm 2 and farm 10. These findings agree with previous research study and also concentration of heavy metal such as Mn, Zn ,Pb, Cr and Cu were increased in recent times (Vikramreddy *et al.,* 2013).

In the study area heavy metals Cu, Cr, V, Zn, Pb, Ni, Co and As level in soil were shown low as compare to permissible limit. The levels of Copper and V in soil normally reflect the concentration in parent and pedogenic process, like Cu in igneous basaltic rocks (90 mg/kg). Composition of the parent material has less bearing on V content of mature, developed soils. Zinc is readily adsorbed by clay minerals, carbonates. Moreover the level Zn in soil is within permissible levels, which indicate its normal concentration and reflect the background value in soil. The main source appears to be the geogenic contribution of Zn in farm soil. The present findings showed the higher level of Zn and Cu. the result of same was on par with the findings of Vikramreddy *et al.,* 2013. Most of the farming soil showed higher concentration of Ba compare with maximum permissible limit. Barium waste may be released to air, soil and water during industrial operations. Barium is released into the air during the mining and processing of ore and during manufacturing operations. All barium compounds that are water or acid soluble are poisonous (ATSDR, 2000).

6.2 Soil physical, physico-chemical, chemical and biological properties:

Based on the study soil bulk density was highest in farms 3 and 8 (1.25 g/cm^3) and lowest was in farm 5 (1.05 g/cm^3). Volume of soil particle was high in far 2 (27.3 cm^3) lowest was in farm 1 (23.4 cm^3). Particle density was high in farm 4 (2.4 g/ cm^3), lowest was in farm 2 (1.8 g/cm^3). Water holding capacity was high in farm 3 (80.4 %) and lowest in farm 1 (62.3). pH range was high in farm 8 (8) lowest in farm 10 (6). ECE was higher in farm 5 (0.43 mS/cm) lower was in farm 1 (0.069 mS/cm) (table 4).

The total amount of nitrogen (3.57 g/kg), total phosphorus (2.38 g/kg) and No3-N (2.17) and SO_4 - S (1.14 mg/kg) were present higher amount in farm 3 soil. Total potassium (62.2 g/kg), NH4-N (1.1 g/kg), extractable phosphorus (0.62 g/kg) and extractable sodium were present higher amount in farm 9 soil. Farm 2 soil was containing high amount of extractable calcium (2. 04 mg/kg) (table 5). Amount of organic carbon was high in farm 6(9.6 g/kg) Activity of β- glucosidase (79.18 mg p-NP g^{-1} soil h^{-1}) (figure 2), bacterial population (45 CFU $g^{-1}\times10^7$) (figure 3) and amount of soil respiration (figure 6) (6.9 CO_2 (mg).100 g of $soil^{-1}day^{-1}$) were high in farm 3 soil. Population of fungi (54 CFU $g^{-1}\times10^4$) was high in farm 6 soil (figure 4) and Actinomycetes (CFU $g^{-1}\times10^5$) was high in farm 9(figure 5).

Among 10 farms, farm 3, 6 and 9 were applying more amount of bio fertilizers, farmyard manure and vermicompost. These factors were enhanced the amount total N, total P, NO_3-N, SO_4- S and microbial population in soil (Padmavathy and Poyyamoli 2011). β-glucosidase was common and major enzyme in agriculture soils. Compare to 10 farms soils organically managed farming soil showed highest activity of β-glucosidase enzyme. Microbial degradation and organic matter deposition were improved the β-glucosidase activities in soil. These enzyme properties can be used as a good biochemical indicator for measuring ecological changes resulting from soil acidification (Acosta-Martı´nez *et al.,* 2003). Soil respiration was significantly higher in organic farms 3, 6 and 9. This indicates a higher soil microbial activity due to the addition of liable organic matter to the soil because of the stimulation of heterotrophic micro-organisms (Ademir *et al.,* 2009).

6.3. Relationship between soil biological and chemical properties:

In the research investigation the results showed that β-glucosidase activity and soil respiration were significantly correlated with total N, total P, total K, organic carbon, NO_3-N and SO_4-S. Bacterial population was significantly correlated with NO_3-N, extractable P and extractable K. Fungal and actinomycetes populations were significantly correlated with total P, total K, organic carbon and extractable K. There were no correlation with biological parameter with NH_4-N and extractable Na. Soil organisms contributed to wide range of functions are essential for all ecosystem. This function includes C, P, N and S cycling and turn over soil organic matter by enhancing the efficiency of plant available nutrients and utilized by crops (Hassink 1994). β-glucosidase enzyme is produced by a wide range of soil organism and their activity mainly links to the amount of soil organic matter. This enzyme was characteristically used as a soil qualitny indicator and may give reflection of past biological activity and soil organic matter (Ndiaye *et al.,* 2000).

6.4 Effects of heavy metals on soil physical, chemical and biological parameters:

Heavy metal Ni was significantly positive correlated with total N, total P, organic carbon, SO_4-S, β-glucosidase and soil respiration. Cu was significantly positive correlated with Electrical conductivity, total P and organic carbon. Pb was significantly positive correlated with total and SO_4-S. Water holding capacity and total P were significantly positive correlated with Manganese. Total N, total P, NO_3-N, bacterial population and fungal population were significantly correlated with V. Cobalt was significantly positive correlated with total N and total P. Sr was significantly positive correlated with water holding capacity, total K, extractable K and soil respiration. Lastly Ba heavy metal positively correlated with more number of soil parameters Viz, water holding capacity, total K, organic carbon SO_4-S, extractable K, β-glucosidase activity, soil respiration, fungal and actinomycetes populations. Cr and Zn metals were not showed significant positive correlation with any soil parameters. Application of excess amount of nitrate and DAP fertilizers, Cu based fungicides and pesticides main reason to enhance the level of heavy metal in agricultural soils. In other hand Organic materials, such as farm manures, composts contain higher concentration of trace elements than most agricultural soils. The use of bio-solids and composts increases total amount of Cu, Zn, Pb, Cd, Fe and Mn in soils (Hariprasad and Dayananda 2013).

7. Conclusion

The present study showed that farm 3 soil was containing higher level of plant available nutrients, sol microbial population and β-glucosidase activity. Microbial activities was the main factor for enhance the plant available nutrients in agriculture soil thorough the process of organic matter decomposition and mineralization. Heavy metals such as, Cu, Cr, V, Zn, Pb, Ni, Co and As concentration were lower than permissible limits. However enrichment levels of these metals were high in current years. Most of the farming soil showed metals Ba and Sr were higher than maximum permissible limit. It can be concluded that all farming soils affected by Ba and Sr metals toxicity. The enrichment of these metals in agriculture soil samples conforms their higher input of synthetic fertilizer and fungicides such as urea, DAP, MOP and complex fertilizers.

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