Chapter I

Results and Discussion
Soil health can be defined as “the continued capacity of soil to function as vital living system within ecosystem and land use boundaries, to sustain biological productivity, promote the quality of air and water environments and maintain plant, animal and human health” (Doran et al., 1997). Bio-diversity or biological diversity has been defined as “the quantity and structure of the biological information contained in hierarchical living ecosystems” (Wilson and Peter, 1988; Blondel, 1995). An increase in bio-diversity of ecosystem or community is, in general associated with greater stability in productivity (Tilman et al., 1996).

4.1 RAINFALL DATA

Data for rainfall in mm was obtained from meteorological department of India (Mausam Bhavan) for the experimental tenure. In the year 2007 the rainfall in May month and January month was 63.7 and 11mm respectively. In the year 2008 the rainfall in the months of May month and January month was 98.3 and 50.5mm respectively. In the year 2009 the rainfall in the May month and January month was 10.9 and 7.3mm respectively. Rainfall was recorded high during the May months with highest in May 2008. Low rainfall was seen in January month except in 2008 January. There was significant difference in January and May months except in 2009 which showed very little but almost equal rainfall in May and January months respectively (Fig-3).

4.2 PHYSIO-CHEMICAL PROPERTIES OF SOIL

Chamba field and natural soil both were found to be sandy loam in nature. Particle size distribution of the cultivated soil was: sand 60 percent, silt 26.5 percent and clay 13.5 percent. Particle size distribution in the uncultivated soil was: sand 55 percent, silt 30 percent and clay 15 percent. Dry matter and soil water content of the cultivated soil were 93.15 ± 0.69 percent and 6.85 ± 0.69 percent respectively. Dry matter and soil water content of uncultivated soil were 72.92 ± 0.32 percent and 27.08 ± 0.32 percent respectively.

Soil pH of Chamba soil was slightly alkaline in nature measuring 7.56 ± 0.01 in the cultivated soil measuring 7.3 ± 0.06 in the uncultivated soil.
The organic matter (percent g\(^{-1}\) soil) in the Chamba cultivated soil was 0.45 ± 0.3 percent and in the uncultivated soil was 1.05 ± 0.15 percent. Organic matter was found to be higher in the cultivated soil in comparison to the uncultivated soil.

Exchangeable Potassium (Kg ha\(^{-1}\)) in Chamba cultivated soil was 301.27 ± 3.5 Kg ha\(^{-1}\) and in the uncultivated soil was 158.72 ± 2.32 Kg ha\(^{-1}\). Quantity of Exchangeable Potassium was comparatively less in uncultivated soil than in cultivated soil.

Exchangeable Sodium (ppm g\(^{-1}\) d wt\(^{-1}\) of soil) in Chamba cultivated soil was 9.49 ± 0.11 ppm g\(^{-1}\) d wt\(^{-1}\) of soil and in uncultivated soil was 10.53 ± 0.33 ppm g\(^{-1}\) d wt\(^{-1}\) of soil. Physio-chemical properties of Chamba cultivated and uncultivated soil have been summarized in Table 2.

### 4.3 SOIL MICROBIAL ANALYSIS

#### 4.3.1 Bacterial population

Bacterial population measured by cfu counting in Thornton’s medium was found to be higher in the cultivated field as compared to the uncultivated field for all the three years i.e. 2007, 08 and 09. Bacterial population was higher in May month as compared to that in January month for two years i.e. 2007 and 2009. In the year 2008 cfu count was slightly higher in the month of January than in the May month (Fig 4). Bacterial population showed a strong positive correlation to the amount of rainfall in Chamba valley in both the cultivated field as well as the uncultivated field (Pearson correlation coefficient 0.978 and 0.00 respectively). Also positive correlation was seen in bacterial population and dehydrogenase activity in both cultivated as well as uncultivated soil (Pearson correlation coefficient 0.847 and 0.039 respectively).

#### 4.3.2 Microbial biomass

Microbial biomass measured as the total genomic DNA in soil was higher in cultivated soil as compared uncultivated soil. Among cultivated samples it was seen to be higher in May month soil as compared to January month soil. Difference between May month and January month in cultivated soil was much higher than difference in May month and January month in uncultivated samples (Fig 5), thus indicating that there has been a positive anthropogenic effect of organic farming on the soil quality.
Fig 3: Rainfall recorded in mm of two months i.e. May and January in Chamba region during the experimental tenure.

Fig 4: Bacterial population enumerated by plate count technique in cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively.

C: Cultivated soil, U: Uncultivated soil
4.3.3 Soil enzymes

4.3.3.1 Dehydrogenases activity

Dehydrogenases are intracellular, oxido-reductase enzymes and, theoretically, can function only within viable cells. DHA of soil is a result of activity of different dehydrogenases. DHA is thus an indicator of biological redox system and can be taken as a measure for intensity of microbial metabolism in soil.

Dehydrogenase activity (DHA) was higher in cultivated soil as compared to uncultivated soil in all the three year readings. Lowest DHA levels were seen during the January month in all the three years except in 2009 (Fig 6). DHA was seen to be positively correlated to bacterial population enumerated by cfu counting using plating technique in both cultivated as well as uncultivated soil samples (Pearson correlation coefficient 0.875 and 0.039 respectively).

4.3.3.2 Urease activity

Urease catalyzes the hydrolysis of urea to CO$_2$ and NH$_3$. Urease activity was higher in cultivated soil as compared to uncultivated soil in most of the readings, although there were fluctuations seen in this pattern during May month 2008 reading. Lowest urease activity was seen in January months as compared to May months. In 2009 there was no significant difference between January month and May month probably because of equal amounts of rainfall in the two months (Fig 7). Urease activity in cultivated soil was positively correlated to nitrate reductase activity and amount of rainfall (Pearson correlation coefficient 0.831 and 0.848 respectively). Urease activity showed strong positive correlation to arginine deaminase activity in uncultivated soil (Pearson correlation coefficient 0.886).

4.3.3.3 Nitrate reductase activity

Nitrate reductase indicates anaerobic nitrate reduction. Its activity was higher in May month samples as compared to January month samples in all the readings of the three consecutive years studied. Not much difference was observed between cultivated and uncultivated samples (Fig 8). Nitrate reductase activity strongly correlated with
urease activity and amount of rainfall in the cultivated soil (Pearson correlation coefficient 0.831 and 0.803 respectively).

### 4.3.3.4 Arginine deaminase activity

Ammonia released from arginine is due to activity of microbes. Therefore this method is used to study microbial activity. Arginine deaminase activity (ADA) was higher in uncultivated soil as compared to the cultivated soil in all the three year data i.e. 2007, 2008 and 2009. ADA was lowest in the January month in all the three consecutive years i.e. 2007, 2008, 2009. Overall ADA showed consistent pattern in cultivated as well as uncultivated soil over a period of three years i.e. highest activity was seen in May months and least activity was seen in the January months (Fig 9). ADA showed a positive correlation to urease activity in cultivated as well as uncultivated soil (Pearson correlation coefficient 0.706 and 0.886 respectively) whereas it showed a positive correlation to the amount of rainfall in uncultivated soil (Pearson correlation coefficient 0.715).
Fig 5: Microbial Biomass assessed by total DNA isolated from cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively.

Fig 6: Dehydrogenase activity in cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively.

C: Cultivated soil, U: Uncultivated soil
Fig 7: Urease activity in cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively.

Fig 8: Nitrate reductase activity in cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively.

C: Cultivated soil, U: Uncultivated soil
Fig 9: Arginine deaminase activity in cultivated and uncultivated soil in the month of May and January months from cultivated and uncultivated soil respectively

C: Cultivated soil, U: Uncultivated soil
The aim of this study was to assess the temporal effect for two months over a period of three years and management practices on soil health. In order to achieve the above said objective, the soil microbial biomass (bacterial population), and soil enzyme (urease, nitrate reductase, arginine deaminase and dehydrogenase) activities were measured in the uncultivated soil and cultivated soil that was intervened by humans. The farmer had maize plantation used organic manure in cultivated field and uncultivated field had natural vegetation on it.

4.4 RAINFALL

Data on rainfall in mm of Chamba valley was obtained by the website maintained by the Meteorological Department of India. Soil moisture levels generally follow the precipitation pattern.

Theoretically, a general consideration is that soils with high soil moisture content should possess high dehydrogenase activity because SMC enhances the microbial activities and dehydrogenases exists in soils as integral parts of intact cells. In our study the total rainfall in Chamba significantly correlated with soil bacterial count. Similar results that soil moisture is an important factor controlling the microbial community structure and activity have been reported by Diaz-Ravina et al, 1995; Frey et al, 1999 and Rigobelo, 2004. Also in our study total rainfall in Chamba correlated with soil dehydrogenase activity and soil nitrate reductase activity. Similar findings have been reported by Subrahmanyam, 2011 and Brzezinska et al, 2001 who said that SMC is positively correlated with its corresponding dehydrogenase activity.

4.5 BACTERIAL POPULATION

Soil bacteria are abundant, diverse, and play important roles in biogeochemical cycles that drive terrestrial ecosystems (Gans et al, 2005).

Bacterial population measured was found to be higher in the cultivated field as compared to the uncultivated field. The present study investigation revealed that the soil bacterial population was higher for cultivated soil in comparison to uncultivated or natural soil for all the three years 2007, 2008 and 2009. This could be due to the many reasons. Regular ploughing and tilling in the cultivated fields, facilitates proper aeration
to even the lower layers of the soil. The nutrient supplements added in cultivated fields can also be accounted for such an observation. Tu, 1995, reported similar observations.

Bacterial population was higher in May month as compared to that in January months for two years i.e. 2007 and 2009. Similar result of higher bacterial population in the summer months has also been reported by Lipson, 2001; Shang, 2005. In the year 2008 cfu count was slightly higher in the January month than in the May month. This may be attributed to the increased rain in January month in Chamba in the year 2008.

Bacterial population showed a strong positive correlation to the amount of rainfall which invariably decides the soil moisture in both the cultivated field as well as the uncultivated field (Pearson correlation coefficient 0.978 and 0.00 respectively). Similar positive correlation between soil moisture and microbial activity was observed by Diaz-Ravina et al, 1995; Frey et al, 1999 and Rigobelo, 2004. Also positive correlation was seen in bacterial population and dehydrogenase activity in both cultivated as well as uncultivated soil (Pearson correlation coefficient 0.847 and 0.039 respectively) and with nitrate reductase activity in both cultivated as well as uncultivated soil (Pearson correlation coefficient 0.847 and 0.824 respectively). Frankenberger, 1982 saw correlation between dehydrogenase activity and microbial respiration as measured by CO$_2$ evolution in soils. The activity and diversity of soil micro-organisms are directly influenced by changes in the soil environment. Changes in soil water content (Bossio and Scow, 1998), pH (Fierer and Jackson, 2006), soil type and soil properties (Wu et al, 2008), plant diversity and composition (Carney and Matson, 2006) have all been shown to influence the composition of soil microbial communities.

### 4.6 MICROBIAL BIOMASS

The total genomic DNA in soil was expressed in terms of soil molecular microbial biomass. This was found to be higher in cultivated soil as compared uncultivated soil. Seasonal changes in soil moisture, soil temperature and available residue could have a strong effect on soil microbial biomass and its activity (Diaz-Ravina et al, 1995). Among cultivated samples it was found to be higher in May month soil as compared to January month soil. Similar findings of more microbial biomass in
summer months have been confirmed by Lipson 2001, Shang 2005. In uncultivated samples the microbial biomass was more or less similar for both May and January months.

4.7 SOIL ENZYMES

The enzyme activities have often been used as indices of microbial activity and soil fertility (Kennedy and Papendick, 1995; Zahir et al, 2001). Soil enzymes derived primarily from soil microbes are important due to the fact that they participate in elemental cycling and decomposition of organic residues and are considered fundamentally good indicators for soil quality (Abdalla and Langer, 2009; Kizilkaya et al, 2007; Venkatesan and Senthurpandian, 2006; Caldwell, 2005). Therefore, the study of soil microbial biomass and their potential activity is important for understanding early changes in biological quality of soil following changes in the land management (Palma et al, 2000).

Skujins (1976) noted that seasonal variations in enzymatic activities were generally small, once the enzymes become stabilized in soil they manifest resistance to humidity, temperature and to various environmental changes. Conversely, our results were consistent with findings reported by Khaziyev, 1977 and Salfeld and Sohtig, 1977 that there were considerable temporal variations in enzymatic activity.

In our study it was observed that all the four soil enzymes showed higher activity in response to higher temperatures i.e. during the May months. Such increases of the soil enzyme activities in response to higher temperatures have also been observed in several experiments in tropical and temperate non-mediterranean ecosystems (Tscherko et al, 2001; Fey and Conrad, 2003; Fenner et al, 2005).

4.7.1 Dehydrogenase

Dehydrogenases are intracellular enzymes and theoretically can function only within viable cells (Alef and Nannipieri, 1995; Stepniewska et al, 2007. Dehydrogenase activity in soil is very sensitive to various natural and anthropogenic factors like soil aggregation, soil aeration status (Brzezinska et al, 2001), organic content (Gajananda, 2007; Tirol Padre et al, 2006) devegetation (Bastida et al, 2006), agricultural management...
(Truu et al, 2008), addition of pesticides (Stepniewska et al, 2007), insecticides (Singh and Kumar, 2008) and heavy metal combined pollution (Gao et al, 2010).

In our study DHA was found to be higher in cultivated soil as compared to uncultivated soil in all the three year readings. Contrary to our result DHA was found higher in undisturbed forest soil by Matinizadaeh, 2008.

DHA varied between seasons. In our study it was least during January months in all the three years except in 2009. Similar result was seen by Ralte et al, 2005. Contradictory result i.e. highest DHA in winter was reported by Montero et al, 2004. Montero et al also reported another peak of DHA in summer months. Similar high DHA in summer months was also observed by Rastin et al, 1998; Kaiser and Heinemeyer, 1993. Kang et al, 2009 said that soil DHA increased with increase in soil temperature. Contrary to our study Boerner et al, 2005 reported no observable seasonal effects.

In our study DHA was seen to be positively correlated to bacterial population in both cultivated as well as uncultivated soil samples (Pearson correlation coefficient 0.875 and 0.039 respectively). Consequently, DHA has been measured to evaluate microbial activity (Tabatabai, 1994; Quilchano and Maranon, 2002). In our study a positive correlation was noticed between DHA and amount of rainfall. Since soil moisture levels generally follow the precipitation pattern thus the amount of rainfall is in turn related to the soil moisture content. Similar findings were observed by Subrahmanyam, 2011 in alluvial soils.

4.7.2 Urease

Urease catalyzes the hydrolysis of urea to CO$_2$ and NH$_4$. It is an important enzyme in soil that mediates the conversion of organic nitrogen to inorganic nitrogen by hydrolysis of urea to ammonia. Urease is released from living and disintegrated microbial cells, and in the soil it can exist as an extracellular enzyme absorbed on clay particles or encapsulated in humic complexes (Mulvaney and Bremner, 1981; Paulson and Kurtz, 1969).

In our study urease activity was mostly higher in cultivated soil as compared to uncultivated soil, although there were fluctuations seen in this pattern.
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In our study lowest urease activity was seen in January months. Urease activity was higher in May months. Similar results were seen by Miller and Dick, 1995 who also recorded peak urease activity during spring and summer and lowest activity during winter. Tiwari and Mishra, 1995 recorded peak urease activity during autumn and trough in winter. Vardavakis, 1989 and Palma and Conti, 1990 also reported peak urease activity during summer when moisture and temperature were most favourable. Contrary results i.e. no correlation of urease with climatic variability was seen by Montero et al, 2004.

In our study urease activity in cultivated soil correlated with nitrate reductase activity and amount of rainfall in cultivated soil (Pearson correlation coefficient 0.831 and 0.848 respectively). Palma and Conti, 1990 reported that the seasonal fluctuations in the urease activity in the soil are influenced by climate and soil type. Similar to our findings Uzun et al (2011) reported positive correlation of urease activity to soil moisture content. Urease activity showed strong positive correlation to arginine deaminase activity in uncultivated soil (Pearson correlation coefficient 0.886).

4.7.3 Nitrate reductase activity

Nitrate reductase activity in soil indicates anaerobic nitrate reduction. In the process of denitrification, dissimilatory nitrate reductase catalyses the first step by reducing $\text{NO}_3^-$ to $\text{NO}_2^-$. 

In our study not much difference was observed between nitrate reductase activities in cultivated and uncultivated soil in all the readings of the three consecutive years studied.

Considering the temporal variation among samples it was seen that nitrate reductase activity was highest in May month followed by January months. This pattern was not seen in 2009.

Nitrate reductase activity strongly correlated with urease activity and amount of rainfall in the cultivated soil (Pearson correlation coefficient 0.831 and 0.803 respectively).
4.7.4 Arginine deaminase activity or arginine ammonification activity

Very little is known about arginine deaminase enzyme which catalyzes the irreversible hydrolysis of arginine to citrulline and ammonia. This enzyme belongs to the family of hydrolases, those acting on carbon-nitrogen bonds other than peptide bonds, specifically in linear amidines. Ammonification is defined as ammonia liberation from nitrogenous compounds which are used as C or N sources.

In our study ADA was higher in uncultivated soil as compared to the cultivated soil in all the three years i.e. 2007, 2008 and 2009. Similar results have been reported by Kumar and Singh, 2008.

ADA was lowest in the January months and highest in May months in all the three consecutive years i.e. 2007, 2008, 2009.

ADA showed a positive correlation to urease activity in cultivated as well as uncultivated soil (Pearson correlation coefficient 0.706 and 0.886 respectively) whereas it showed a positive correlation to the amount of rainfall in uncultivated soil (Pearson correlation coefficient 0.715). Alef and Kleiner, 1987 reported ADA or arginine ammonification to be strongly correlated to soil respiration and carbon content of soil, but not or only poorly with soil pH, ammonia content, percentage clay or the number of micro-organisms. It is also reported that ADA or arginine ammonification has a significant correlation to soil microbial biomass and biochemical activities (Davis et al, 1973; Alef and Kleiner, 1987). No such correlation was seen in our studies.