Pak. J. Weed Sci. Res., 19(3): 305 - 314, 2013

NITROGEN FIXATION BY NON LEGUMINOUS PLANT SEA BUCKTHORN IN SEMI ARID CLIAMATIC CONDITIONS OF GILGIT-BALTISTAN

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ABSTRACT

The study aims at quantification of nitrogen fixation by a non leguminous medicinal plant i.e. seabuckthorn (*Hippophae rhamnoids* L.) at different ages and depths particularly its effects on others physio-chemical parameters of the soils at Jalalabad Village, District Gilgit, Baltistan. The results showed that contents of Nitrate-Nitrogen (NO_3-N) of the sampled soil increased near root nodules and in aged plants, while decreased with increasing depth and in younger plants or forest. Average maximum value of NO_3-N was 7.96 ppm at 12 cm depth and minimum value of 2.41 ppm was at 25 cm depth; while at 50 cm, it was slightly higher than 25 cm depth sample which was 2.43 ppm. Simlarly, organic matter of sampled soil increased with age of forest and decreased with increase in depths and in younger plants or forests, where average maximum value was recorded 1.65% at 12 cm depth in 10 year age forest, the percent organic matter at 25 cm depth was found to be median as compared to 12 cm and 50 cm depth where average percent organic matters was 1.10% and average minimum value was recorded 1.06% in soil sample at 50 cm depth in 8 years age forest. The pH value of the sampled soil increased with increasing soil depth average. The pH value was minimum 7.32 pH at 12 cm depth and maximum 7.55 pH at 25 cm depth. At 50 cm, it was sligtly lower to 25 cm similarly pH value decreased with the age of forest compared to control. It was maximum 7.55 in 10 years forest and minmum 7.41 pH in 8 year forets.after 8 years pH slightly increased to 7.55 to in soil for 10 years forest. Electrical conductivity (EC) value of the sampled soil decreased with increasing in soil depth and the age of Sea buckthorn forest, Average value was minimum at 25cm depth (0.24) dsm^{-1} and maximum was (0.42) dsm^{-1} at 12 cm depth. At 50 cm it was slightly higher to 25 cm deep soils. Similarly, It was maximum 0.33 dsm^{-1} in 6 years forest and minimum 0.23 dsm^{-1} in 10 years SBT forest.

Key words: *Hippophae rhamnoids,* organic matter, soil depth, age of forest, pH.

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INTRODUCTION

Seabuckthorn (*Hippophae rhamnoides* L.) is a wild tree or bush and is widely distributed throughout the temperate zones of Asia and Europe and throughout the subtropical zones of Asia at higher altitudes. It is a deciduous, thorny and N fixing shrub/small tree 2-6m high, growing widely on riversides indicating that it is a water loving plant and growing at altitudes up to 5200 m. Sea buckthorn can resist temperatures up to -43^{o}C and can withstand 40^{o}C as well. Some species grow well in regions that only are a precipitation of about 300 mm while others can endure inundation, and it grows well in sandy, stony, and saline soils which is a unique feature of this giant plant.

A natural sea buckthorn forest can yield 150-1500 kg of berries ha^{-1}. Its small orange-colored fruit is a store house of vitamins and important bio active substances. The vitamin C content is 5-100 times higher than any other fruit or vegetable known. Its pulp and seeds contain high quality oil, which is regarded to be very important for its medicinal value. Thus, the sea buckthorn fruit is being used as a raw material for producing food, medicines and cosmetics. In addition, the sea buckthorn plant is a good source of fire wood. In a six-year old sea buckthorn forest, each hectare can produce 18 tons of firewood, equal to nearly 12.6 tons of standard coal. The leaves of sea buckthorn have been proved to contain 11-22% of crude protein and 4-6 percent of crude fat; therefore, the leaves of sea buckthorn are good for forage. Sea buckthorn is a deciduous thorny bush type plant belongs to Elaegnacae the family of Russian olive (*Eloigns anguestifolia*).

There are several species and sub-species of sea buckthorn including *Hippophae goniocarpa, H. gyantsensis, H. litangensis, H. neurocarpa, H. rhamnoides* L., *H. salicifolia* and *H. tibetana*. Only one sub-species *H. rhamnoides turkistanica* was found in Gilgit-Baltistan. It is a dioecious plant and female bears pea size yellow, greenish yellow, orange and red berries. These berries develop directly from calyx without giving flowers. The Seabuckthorns are deciduous shrubs that typically range from 0.5 to 6 m in height with equivalent spread, but may reach up to 18 m in central Asia. The staminate trees are more erect than the spreading pistillate trees. It naturally tends to sucker forming thickets if not properly maintained. They can survive low temperatures, and are both drought and salt tolerant. The branches of seabuckthorn are dense, stiff, and very thorny with both terminal and auxiliary twig spines. The linear or lancelets shaped leaves, which are 3 to 8 cm long and less than 7 mm wide, are dark grey-green on the upper surface and a distinct pale, silvery-grey on the lower surface. Seabuckthorn is dioecious; with separate male and female plants. Pollination of the female flowers occurs in mid-May, and is entirely dependent on wind to spread pollen from the male flowers. Fruit ripening occurs about 100 days after pollination.

Sea buckthorn fruit can vary in both shape and colour, but are typically globosely to egg-shaped berries ranging from yellow to bright orange in colour. The combination of fruit shape and size, together with the contrast between the colour of the fruit and leaves, contributes to the ornamental value of this plant. It is native from northwestern Europe, through central Asia to the Altai Mountains to western and northern China and the northern Himalayas Agricultural Zone.

Sea buckthorn as Nitrogen fixer

Sea buckthorn fixes nitrogen in its root nodules. The roots of this plant spread both vertically and horizontally. The tap roots are 4m long where as horizontal roots are 10m, which help in binding the soil and control soil erosion, thus it is named as plant engineering. Nitrogen can be fixed through this plant by its roots in which elemental nitrogen is added in soil by root nodules i.e. *Rhizobium*, this bacterium fix 180 kg per ha nitrogen annually in soil (180kg ha^{-1} $year^{-1}$). It improves soil fertility by adding Phosphorus and organic matter in the soil in addition to nitrogen.

Classifications

Common name	Seabuckthorn
Local name	Buru
Scientific Name	*Hippophae Rhamnoides* L.
Family	Elaeagnaceae
Flowering period	Spring
Parts used	Leaves, branches,berries and stems.

MATERIALS AND METHODS

Sampling methods and tools

Representative soil samples can best be obtained by using a core sampling tool. The use of a proper sampling tool is essential for sampling to depths below 15 cm. Take soil cores from 0-15 cm at each of the 3 to 4 sampling sites. For improved nitrogen and sulfur evaluation or where problem soils are encountered, separate samples should be taken from the 0-12 cm, 12-25 cm, and 25-50 cm depths at the same 3 sites and 1 control / barren land.

Sample collection

Twelve composite samples were collected randomly from three different sites and one control (barren land area) from Jalalabad village of district Gilgit, using the standard technique given in BARD manual. Samples were collected from depths i.e., 12cm (furrow slice), 25cm and 50cm from three different age forests viz., 10, 08, and 06 years, using soil Augar. Collected samples were air dried and mixed thoroughly to prepare composite samples, and stored in polythene bags, tagged with key information for lab analysis.

Laboratory analysis

Laboratory analysis of the sampled soil were measured for various physical and chemical parameters such as soil organic matter, Nitrate-Nitrogen (NO_3-N), pH, Electrical conductivity (EC), and soil texture were analyzed in the Soil Sciences Laboratory of the Karakorum Agricultural Research Institute for Northern Area (KARINA) as per the standard methods described by Page *et al.* (1982).

Soil organic matter

We took 1 g air dried soil from the samples into a 500 ml beaker, added 10 ml 1N potassium dichromate solution using a pipette, added 20 ml concentrated H_2SO_4 using a dispenser, and swirled the beaker to mix the suspension. Allowed the suspension to stand for 30 minutes, added about 200ml distill water followed 10ml concentrated Ortho-phosphoric acid using a dispenser and allowed the mixture to get cool. Thereafter, we added 10-15 drops of diphenylamine indicator, added a Teflon magnetic stirring bar and then placed the beaker at the magnetic hearer. We titrated the mixture with 0.5M ferrous ammonium sulfate solution until the colour changed from violet-blue to green. Then we prepared two blanks containing all reagents but no soil and treated them in exactly the same way as the soil suspension (Walkley, 1947).

Nitrate-Nitrogen (NO_3-N)

Weighed 10g air dry soil (2mm) into a 125ml conical flask, added 20 ml extraction solution in a reciprocal shaker for 15 minutes at 180 cycle minutes, with flask kept open, and the Extract was then filtered through Watman No-42 filter paper. Transferred 1ml of the soil Extract to 25ml test tube and added 3ml copper sulphate working solution and added 2ml hydrazine sulphate working solution and added 3ml sodium hydroxide. Mixed and heated in a water Bath at 38^0C for 20 minute, added 3ml colour developing solution for NO_3-N mixture and left it stand at room temperature for 20 minutes. Absorbance at 540 nm waves length and spectro-photometer and then got the readings.

Soil pH

Soil pH was determined using an electrode pH meter in a 1:2 soil: water suspension. Firstwe took 20g of sampled soil and 40 ml distilled water at ratio of 1:2 soils: in a beaker andshaked the mixture in shaker for 30 minutes. The sample was left undisturbed for 20 minutes and then recorded reading using an electrode pH meter (Mc-Keague, 1978).

Electrical Conductivity (EC)

Soil Electrical conductivity was determined using an electrode EC meter in a 1:2 soil: water suspension (Mc-Keague, 1978).

Soil Texture

Took 40g air dry soil sample into a 600ml beaker and added 60ml dispersing solution. Covered the beaker with a watch glass, left it for overnight, transferred the contents of the beaker to a soil stirring cup and filled it up to 3 quarters with water. Stirred the suspension with high speed for 3 minutes using the special stirrer. Rinsed stirring paddle into a cup and allowed stand for 1 minute. Transferred the suspension quantitatively into a filter calibrated cylinder and brought the volume to 1000ml with water and soil textures were determined by Bouycoucos Hydrometer method (Bouyoucas, 1962).

Data analysis

The results of laboratory analysis were statistically analyzed using excel 2007 for simple calculations, monographs, tables and interpretation (Mc-Cullough and Heiser, 2008).

RESULTS AND DISCUSSION

Organic Matter

Percent organic matter of the sampled soil increased with age and decreased with depth (Fig. 1). Maximum value (1.65%) was observed at 12 cm depth in 10 year age forest and minimum value (1.06) was recorded in soil at 50 cm depth in 8 years age forest, similarly, as compared to control.

Variations in the values of organic matter with the depth of soil and age of SBT forest might be leaves on the soil surface compared to lesser organic matter content in deeper soil, which probably is due to less or no manipulation/tillage of soil in SBT forest. The soils are generally low in organic matter (usually <1%) (Whiteman, 1985).

Nitrate-Nitrogen (NO_3-N) (ppm)

Nitrate-Nitrogen (NO_3-N) value of the sampled soil decreased with increasing depth. Average value was minimum at 25 cm depth (2.41 ppm) and maximum (7.96 ppm) at 12 cm depth. At 50 cm it was slightly higher (2.43 ppm) to 25cm depth.

Similarly, with the age of forest Nitrate-Nitrogen (NO_3-N) value slightly decreased in 6 and 8 years and increases at 10 years forest, compared to control. It was maximum (10.32 ppm) in 10 years forest and minimum (1.83) in 8 years SBT forest. After 8 years Nitrate-Nitrogen (NO_3-N) is increased to (10.32 ppm) in soils samples collected from 10 years forest.

Nitrate-Nitrogen (NO_3-N) values change with depth and age of SBT forest is possible, NO_3-N increased with the age of SBT forest is due to the presence of organic matter accumulation is increasing with the age of SBT forest. The leaves of SBT plants are very nutritious and fertilize the land when fall from the plants, these leaves and organic matter contains NO_3 it combines with water and dissolves into the soil

(Di and Cameron, 2002). The older age SBT forest root nodules have more ability to fix atmospheric nitrogen to the ground surface as compared to the younger ones SBT forest root nodules, as 8 to 10 years old sea buckthorn forest can fix 180 kg Nitrogen ha^{-1} $year^{-1}$ (Lu, 1992). NO_3-N decreased with increasing depth at 12 cm depth NO_3-N content is more as compared to depth of (25 and 50 cm) because less percolation and leaching of organic materials to lower depths (25 to 50 cm) probably due to less annual precipitation under the prevailing semi arid climatic conditions. The Nitrate-Nitrogen cannot go deeper into the soil only some of the NO_3-N is goes through percolation and leaching (Robbins and Carter, 1980).

Soil pH

The pH of sampled soil increased with the age of the forest, It was maximum (7.55) in 10 years SBT forest and minimum (7.41) in 8 years forest. After 8 years, pH slightly increased to 7.55 in soil for 10 year forest. And the pH of sampled soil also increased with increasing soil depth. Average value was minimum at 12cm depth (7.38) and maximum value was (7.55) at 25cm depth, at 50 cm it was slightly lower to 25 cm depth. pH value changes with depth and age of SBT forest is possibly due to acidic parent material of soil, comparatively higher decomposition of organic residues in 8 year forest soil less percolation and leaching of organic materials to lower depths (25 to 50 cm) probably due to less annual precipitation under the prevailing semi arid climatic conditions (Johnson and Raun, 1995)

Electrical Conductivity (EC)

Electrical conductivity (EC) value of the sampled soil decreased with increasing in soil depth and the age of SBT forest. Average value was minimum at 25cm depth (0.24) dsm^{-1} and maximum was (0.42) dsm^{-1} at 12 cm depth. At 50 cm (0.27) it was slightly higher to 25 cm deep soils. Similarly, It was maximum (0.33) in 6 years forest and minimum (0.23) in 10 years SBT forest.

Electrical conductivity (EC) value changed with depth and age of forest possibly because of the decreased in soil electrical conductivity (EC) with increasing soil depth and age of SBT forest therefore, subsurface features will not be expressed as extensively by electrical conductivity mapping as the same feature if it were located nearer to the soil surface. Electrical conductivity is strongly dependent on temperature. As temperature decreases towards the freezing point of water, soil electrical conductivity decreases slightly, soil pores become increasingly insulated from each other and overall Electrical conductivity declines rapidly. The overall soil of Gilgit-Baltistan is free from salinity problem this is one of the reason for decreasing electrical conductivity (Doerge *et al*., 1999). Another reason is that the existing parent material posses less salt contents.

Table-1. Effect of sea buckthorn leaves on soil physical and chemical properties (Laboratory analysis result of soil sample)

Age (Years)	pH				Electrical conductivity (dsm^{-1})				Nitrate-Nitrogen (ppm)				Organic Matter (%)				Texture		
	12 cm	25 cm	50 cm		12 cm	25 cm	50 cm		12 cm	25 cm	50 cm		12 cm	25 cm	50 cm		12cm	25 cm	50 cm
0	7.71	7.64	7.38	7.58	0.432	0.209	0.488	0.38	3.019	2.722	2.013	2.58	1.10	0.90	0.71	0.90%	sandy loam	sandy loam	sandy loam
6	7.42	7.61	7.51	7.51	0.466	0.281	0.25	0.33	2.739	1.947	2.31	2.33	1.20	0.80	1.30	1.10%	sandy loam	sandy loam	sandy loam
8	6.94	7.66	7.62	7.41	0.508	0.254	0.134	0.30	1.98	1.518	1.98	1.83	0.60	0.95	0.85	0.80%	sandy loam	sandy loam	sandy loam
10	7.44	7.54	7.67	7.55	0.286	0.203	0.209	0.23	24.09	3.465	3.415	10.32	1.78	1.76	1.40	1.65%	loam	sandy loam	sandy loam
	7.38	7.61	7.55		0.42	0.24	0.27		7.96	2.41	2.43		1.17 %	1.10 %	1.06 %		Sandy loam		

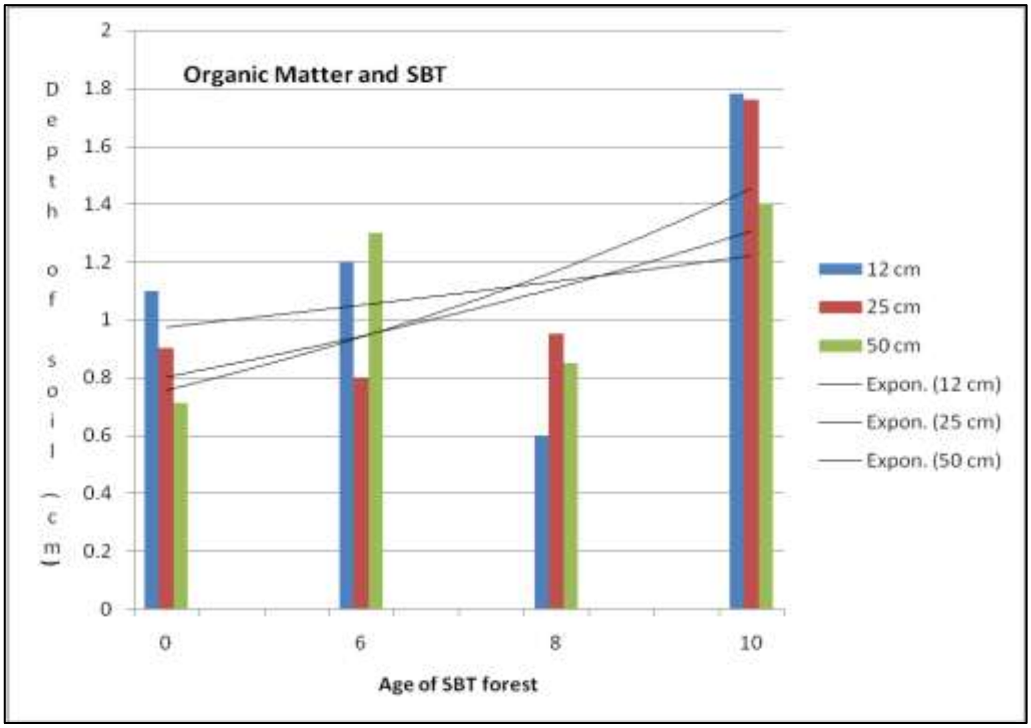

Figure 1. Organic matter and sea buckthorn

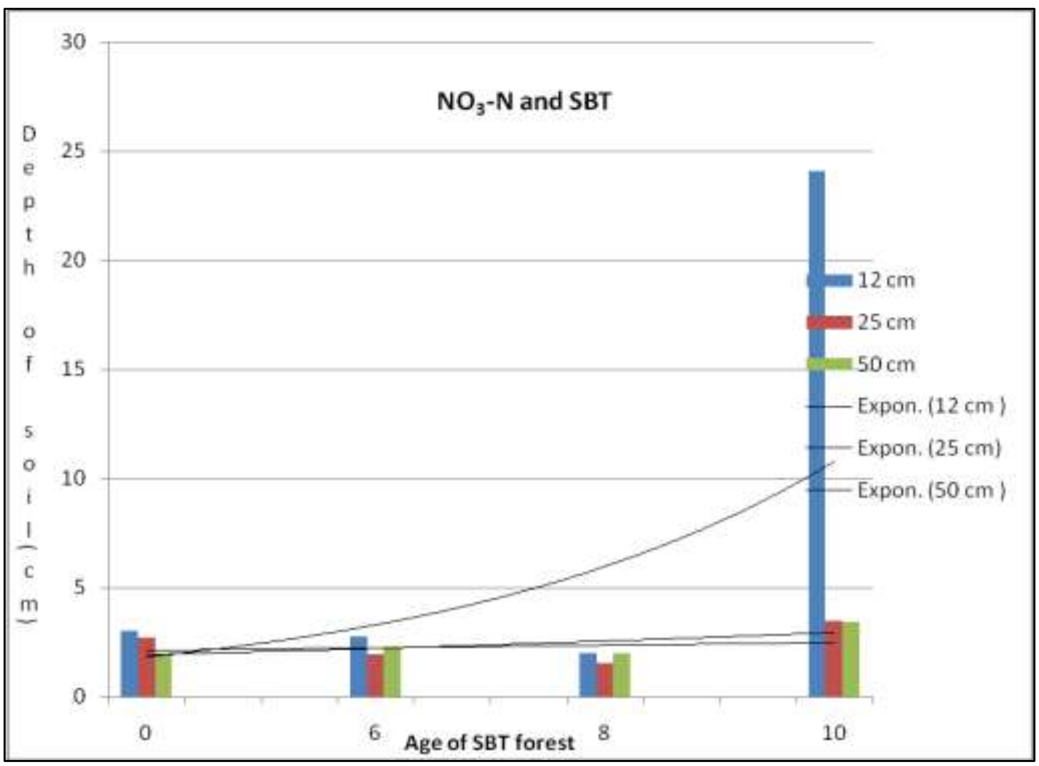

Figure 2. Nitrate-Nitrogen and sea buckthorn

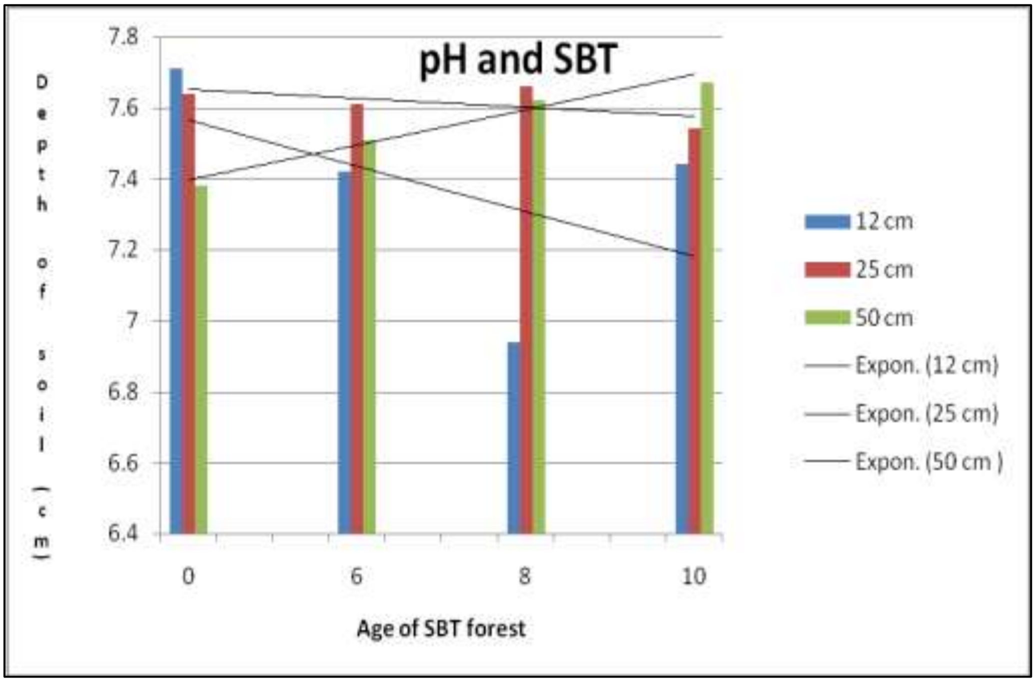

Figure 3. pH and sea buckthorn

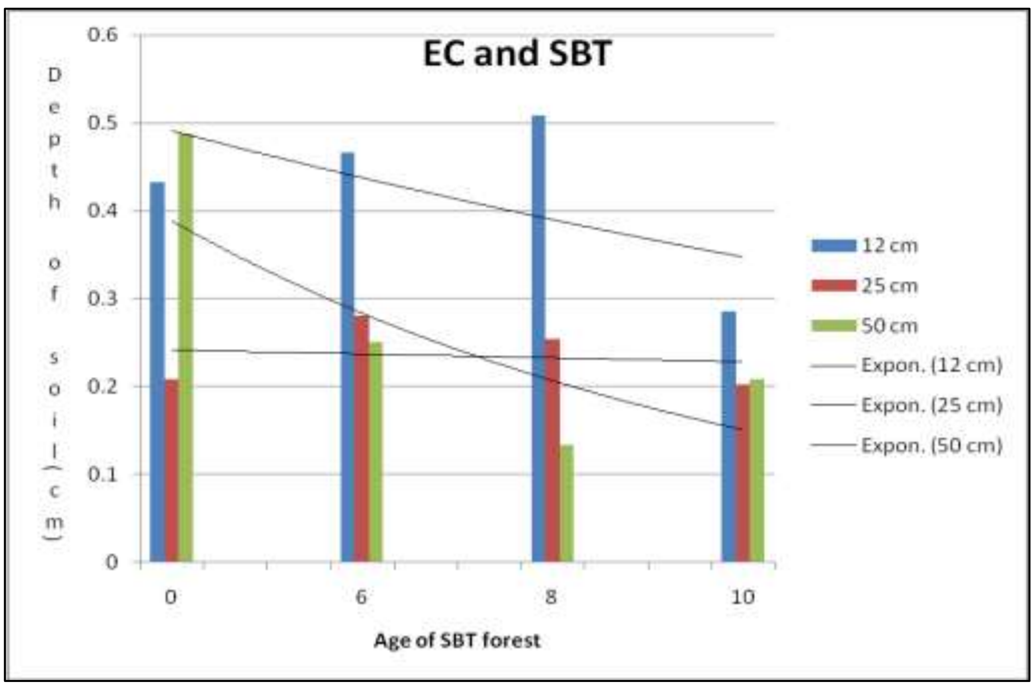

Figure 4. Electrical Conductivity and sea buckthorn

CONCLUSION

Sea buckthorn (*Hippophae rhamnoids* L.) is a non leguminous plant and its importance is for the fixation of Atmospheric Nitrogen to the surface by the help of its root nodules by the symbiotic association with bacteria (azoto-bacter)/Rahizobium bacteria. This bacterium fixes 180 kg ha^{-1} N annually in soil. It improves soil fertility by adding Phosphorus and organic matter in the soil in addition to nitrogen. The older age SBT forest root nodules have more ability to fix atmospheric nitrogen to the ground surface as compared to the younger ones SBT forest root nodules. The Nitrate-Nitrogen cannot go deeper into the soil only some of the (NO_3-N) is goes through percolation and leaching in semi arid climatic conditions. As age of SBT forest increases the amount of (NO_3-N) is also in large quantity, and as the depth increases amount of fix Nitrate-Nitrogen is less. That is why in old age SBT forest has more fixed Nitrogen than the younger one SBT forest. Sea buckthorn leaves also had a significant effect on various soil physical and chemical properties including the organic matter, nitrate-nitrogen (NO_3-N), soil pH, electrical conductivity and soil texture.

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