Agronomic potential of the association *Azolla –Anabaena*

Bhuvaneshwari K and Ajay Kumar

Department of Botany, Banaras Hindu University, Varanasi India
K.bhuvaneshwari01@gmail.com

ABSTRACT

An incubation experiment was carried out in order to investigate the effect of *Azolla* on the physical and chemical properties of the soil in which the soil were treated with *Azolla* at 0,10,30,60 and 90g/kg. The treated soils were incubated in the dark at 25^{o} C for 60 days in the laboratory. The application of *Azolla* increased the soil pH, organic matter, N, P, K, Ca, Mg and Na with rate of *Azolla*. There was reduction in soil bulk density but increased soil porosity.

Key words: Incubation, Azolla, Mineralization, Soil Physical and Chemical Analysis.

INTRODUCTION

Due to the shortage and high price of inorganic nitrogen fertilizers as well as fear of pollution due to their excess use, initiated the research on alternative technology where utilization of synthetic nitrogen fertilizers could be minimized (Singh *et al.,* 1981). Nitrogen fixing crops, composted crop waste and livestock manures are least-cost alternative sources of nitrogen which have been adopted by the farmers on a wide range of situations. Another option, specially for those growing rice in flooded condition or irrigated land is the use of *Azolla. Azolla pinnata* is commonly found in India in ponds, ditches and canals containing stagnant water (Kulasooriya, 1998). *Azolla* is a free floating aquatic water fern which grows at a fast rate doubling its biomass in 3-5 days and fixes atmospheric nitrogen by forming a symbiotic association with the blue-green algae *Anabaena azollae.* This unique property of the plant has drawn the attention of agriculturists and botanists for its utilization in agriculture as organic nitrogen fertilizers for the rice cop. *Azolla* have long been used as both a green manure for rice and as a fodder for poultry and livestock in China and Vietnam (Kamalasamana *et al.,* 2005; Singh, 1971).

Organic matter in the form of green manure and biofertilizers has been found useful instead of the inorganic fertilizers (Nayak *et al.,* 2004; Bhuvaneshwari *et al.,* 2012). Biofertilizers are live formulates of such beneficial microorganisms which on application to the soil mobilize the availability of nutrients by their biological activity in particular and helps to build up the micro-flora and in turn, the soil health in general. It is also affordable and does not cause eutrophication and perturbation of soil (Scheir, 1999). The beneficial effect of the *Azolla* showed that it increases soil organic matter, improved soil and supply fixed nitrogen. After its decomposition, humus is formed which increases the water holding capacity of the soil and promotes aeration and drainage. *Azolla* supply fixed nitrogen and increases the uptake of some nutrient element such as Calcium, Magnesium and potassium (Sinha *et al.,* 2002; Sharland, 1997). The objective of this study is to investigate the effect of *Azolla pinnate* on the physico-chemical properties of the soil.

MATERIALS AND METHODS

Sampling of Soil

The soil was sampled to a depth of 0-15 cm from an uncultivated field of Agricultural farm of Banaras Hindu University Varanasi, India. All the samples were brought to the laboratory, where sieving was done with 2 mm sieve.

Chemical Analysis of the Soil

Part of the seived soil was air – dried and some chemical properties were determined. The pH of the soil was determined using pH meter with glass – column combination electrode in distilled water and 0.01M $CaCl_2$ solution.

The organic carbon content was determined using Walkley and Black Method (1934). The total nitrogen was determined by the Kjeldahl method (AOAC, 1965). Exchangeable K, Ca and Mg were extracted using ammonium acetate, K was determined on Flame photometer (Jackson, 1990) and Ca and Mg by EDTA titration (Thomas, 1983 and Rich, 1952). Soil organic matter was determined by wet dichromate method (Carter, 1993).

Azolla collection, preparation and cultivation

Azolla pinnata was collected from the ditches near the agriculture farm of Banaras Hindu University, Varanasi, India. It was gently washed with tap water and dried with tissue paper and cultured using the IRRI medium. Fresh *Azolla pinnata* was weighed and cultured to obtain enough *Azolla* biomass for the experiment.

Incubation

There were four *Azolla* treatments for mineralization study. Fresh *Azolla* were weighed using an electric meter in 0, 10, 30, 60, 90 g/Kg each in three replicate were mixed with the 5 Kg soil with a hand towel. There were three pots per treatment and the control experiment inclusive. Amended soils were then packed in labeled clay pots, watered with sufficient distilled water. The pots were kept in dark at about 25 °C to incubate for 60 days. Incubated soil samples were collected below the upper oxidized layer (2-5 cm) with a sharp spatula at regular interval of 10, 20, 30, 40, 50 and 60 days of flooding for routine analysis.

Statistical Analysis

Data was subjected to analysis of variance (ANOVA) to determine the treatment effect. The least significance at 5% level of significance was used to compare mean (Steel and Torie 1985).

RESULTS AND DISCUSSION

Table 1 shows the chemical analysis of *Azolla pinnata* used in this investigation (% on dry matter base). The table 2 has data on initial soil analysis used for this experiment. The soil was sandy- loam and it was low in organic matter (OM), available P, marginal in exchangeable K and total N, adequate in exchangeable Ca and Mg and it is slightly acidic (Dawar et al, 2001). Rate of release of nutrients into the soil treated by Azolla amendments (Table 3-6) compared with the control showed that, the different rates used increased the amounts of nutrients over time. The rate of release increased with pericals of experiment that is at 10 and 20 days, the rate of release is lower than what is obtained at 50 days for all the nutrients examined. Table 7 shows the data on the effect of *Azolla* soil physical properties . *Azolla* application tended to reduce the bulk density from 1.35 g/cm^3 in the control to 1.26cm^3 on 90kg^{-1} of the soil and increased its porosity. This might be due to increased organic matter released into the soil. The greater the organic matter content of a soil, the greater the increased porosity, the small the soil compaction, the greater the water content (Defoer *et al.,* 2000).

Table 1: Chemical Analysis of _Azolla pinnata_

Total chl mg/gm/FW	0.65
Protein mg/gm FW	11.5
Organic Carbon %	44.03
Nitrogen %	4.92
Phosphorus %	0.26
C : N ratio %	8.92

Table 2: Analysis of the soil

pH in water (1:2)	7.62
pH in 0.01M $CaCl_2$ solution	6.28
Organic carbon g/Kg	8.7
Total Nitrogen g/Kg	5.0
Available phosphorus mg/Kg	0.57
K (cmol/kg)$^{-1}$	0.52
Ca (%)	0.18
Mg (%)	0.32
Na (cmol/Kg)$^{-1}$	0.58
Sand	58 %
Silt	28 %
Clay	14 %

Table 3: Effect of *Azolla* on soil organic matter (in %)

Treatment	Incubation time (days)				
Azolla g/Kg soil	10	20	30	40	50
0	2.29	2.39	2.39	2.39	2.30
10	3.01	3.17	3.49	3.43	3.91
30	3.21	3.36	3.61	3.53	3.41
60	3.18	3.41	3.71	3.71	3.61
90	3.32	3.52	3.81	3.87	3.81

Table 4: Effect of *Azolla* on soil Nitrogen (%)

Treatment	Incubation Time (days)				
Azolla g/Kg soil	10	20	30	40	50
0	0.16	0.16	0.16	0.16	0.16
10	0.18	0.18	0.31	0.34	0.34
30	0.28	0.24	0.37	0.38	0.38
60	0.37	0.40	0.43	0.51	0.54
90	0.39	0.47	0.50	0.54	0.53

Table 5: Effect of *Azolla* on soil available Phosphorus (ppm)

Treatment	Incubation time (days)				
Azolla g/Kg soil	10	20	30	40	50
0	2.52	2.52	2.52	2.52	2.52
10	2.60	2.73	3.61	4.21	4.23
30	2.87	2.91	3.69	5.29	5.31
60	4.27	4.59	5.29	5.47	5.51
90	4.81	4.92	6.25	5.72	5.73

Table.6 Effect of *Azolla* on soil physical properties (Mechanical analysis)

Treatment Azolla(g/kg)	**Bulk density (gcm^{-1})**	**%Porosity**	**%Soil**	**day**	**% salt**
0	1.35	42	61	21	13
10	1.31	41	61	21	15
30	1.30	41	58	23	17
60	1.28	43	57	23	19
90	1.26	45	56	22	22

Table.7 Effect of *Azolla* on soil chemical properties

Treatment Level (gkg^{-1})	pH	OM (gkg^{-1})	N (%)	P (ppm)	K (c mol kg^{-1})	Ca %	Mg %	Na (mol kg^{-1})
0	6.31	2.42	2.41	0.15	0.49	0.18	0.33	0.58
10	6.31	3.29	3.41	0.24	0.55	0.90	0.46	0.65
30	6.59	3.56	3.57	0.32	0.58	0.99	0.56	0.68
60	6.69	3.69	3.62	0.43	0.64	1.11	0.74	0.69
90	6.21	3.71	3.65	0.47	0.64	1.37	0.93	0.70
LSD(0.005)	0.44	0.22	0.22	0.09	0.01	0.76	0.04	0.00

Soil organic matter (SOM) contents are usually positively related with specific soil properties or process forstering crop growth, such as cation-exchange capacity, rainfall in fliteration or soil structure (Vaulaurve et al 2004). Generally , the less organic matter a soil contains, the weaker its structure and the greater the risk of serious erosion (Defoer et al 2000). Table 8 has data on chemical properties of soil as effected by Azolla for 60 days. Soil incubation leads to increase in soil pH, organic matter (OM), N , P , K, Ca, Mg and Na. There was significant differences ($p>0.05$) among untreated control and other treatments (0 , 10, 30, 60 and 90 gkg^{-1} of Azolla).

Difference between 0g kg^{-1} were not significant for most chemical properties in the soils except with organic matter. There was a significantly higher mean value of nitrogen with soil treated with 90 g kg^{-1}. The significantly high mean value of nitrogen as a result of high N content in Azolla is reported by Singh, 1981 and Bhuvaneshwari 2011. The higher mean value of pH in 60g/kg had shown that Azolla is able to control acidity by raising pH value which in the line with the findings of Kotpal and Bali (2003). The higher significant mean values of small phosphorous in the soil were due to high phosphorous content of Azolla (Michelle and Jude, 1990). It may also be as a result the increase in soil pH values. The significantly high mean values of Ca and K may be due to high mean values of nitrogen which has enhanced their uptake.

Application of Azolla has been found to improve the physical and chemical properties of the soil. These improvements were significant for Nitrogen, Organic matter and other cations (Mg, Ca and Na) released into the soil. Careful management of soils in the tropics with Azolla results in better production of crops since its production is cheap, economical and eco-friendly. Azolla production and utilization is labour intensive and it can be done in farmers farm.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Science and Technology (DST) (WOS)-A, New Delhi, India for providing financial assistance and to the Head of Department Botany, B.H.U., Varanasi for providing the facilities required for carrying out the present investigation.

LITERATURE CITED

AOAC, 1965 *A.O.A.C. Official and Tentative Methods of Analysis.* Association of official analytical chemist. 10th Edn. Washington, DC, U.S.A.

Bhuvaneshwari K, Singh R and Singh PK, 2012. Organic Rice production using Organic manures and Bio-inoculants in an Alkaline soil. *In J Rec Adv Agri* **1**(4):128-134.

Bhuvaneshwari K, 2012. Beneficial effects of Blue-Green Algae and Azolla in rice culture. *Environment Conservation* **13**(1 & 2):1-5.

Carter MR, 1993. *Soil Sampling and methods of analysis.* Canadian Society of Soil Science. Lewis Publishers, London pp: 823.

Dawar ST, Dey T, Saxena S and Singh PK, 2001. Biodiversity of Azolla and Prospects of its genetic improvement in relation to temperature, salinity and Pest infestation In:(Singh PK, Dolly DW, Pabby S, Prasanna R and Arora A. eds*) Recent Advances in Exploitation of Blue Green Algae and Azolla, NCCUBGA*, IARI, New Delhi pp. 129-137.

Defoer T, Buddman A, Toulmin C and Carter SE, 2000. *Building common Knowledge participatory learning and action research (Part 1)* In: Defoer T., Buddman A. (ed) Managing soil fertility in the topics , KIT, Netherlands pp. 208.

Jackson ML, 1990. *Soil chemical Analysis 6th Edition* Prentice Hall India, Pvt. Ltd. New Delhi, India.

Kamalasamana P, Premalatha and Rajamony S, 2005. *Azolla :* A sustainable feed for live stock. *LEISA India* **21**(3):26-27.

Kotpal RL and Bali NP, 2003. *Concepts of Ecology: Environmental and field Biology:* Visual Publishing Company India.

Kulasooriya SA, 1998. Cyanobacteria and *Azolla* as biofertilizer for rice pp. 465- 564, In: Subramaniam G, Kaushik B D, Venkataraman G S.(eds). *Cyanobacterial biotechnology*. Science Publishers, Netherlands.

Nayak S , Prasanna R , Pabby A, Dominic TK and Singh PK. 2004 . Effect of urea, blue green algae and *Azolla* on nitrogen fixing and chlorophyll accumulation in soil under rice. *Biol. Fertile Soils*, **40:**67-72.

Rich CI. 1952, Flame photometric determination of exchangeable Magnesium in soil*. In Soil Science Society of American Journal,* **16**(1):51 – 55.

Scheir S, 1999. Soil degradation. A threat to developing country food security by 2015. *Food, Agricultural and the Environment Discussion paper* 27,*IFRRI* Washington DC.

Sharland RW, 1997. Understanding traditional perceptions of *Azolla,* a means of communicating soil fertility, *ILEIA Newsletter*, **13**(3):23-29.

Singh PK, Panigrahi BC and Satapathy B, 1981. Comparative efficiency of *Azolla,* blue-green algae and other organic matter in relation to N and P availability in a flooded rice soil. *Plant soil*, **62** 35-44.

Singh PK, 19971. Effects of Azolla on the yield of paddy with and without application of N- fertilizer. *Current Science* **46:**642-644.

Sinha SK, Verma DC and Dwivedi CP, 2002. Role of green manures (*Sesbania rostrata*) and biofertilizers (Blue-green Algae and Azotobactor) in rice-wheat cropping systems in the state of Uttar Pradesh, India*. Physiology and Molecular Biology of Plants* **8:**105-110.

Steel RGD and Torie JH 1985, *Principles and procedures of statistics. McGraw- Hill Book Co. Inc. New York, pp . 481.*

Thomas GW, 1983. Exchangeable cations. Pp. 159-164 *In Methods of Soil Analysis part 2. Chemical and Microbiological properties 2nd Ed Agron. No.9*

Vaulaurve B, Sangigo N, Giller K and Merckx R, 2004. Management of Nitrogen fertilizer in maize based, systems in sub humid areas of sub- Saharan Africa. *Agriculture and the Nitrogen cycle. SCOPE (eds) Arvin. Mosier, Keith sysers and John R. Frency pp. 115-127.*

Walkley A and Black IA, 1934. An examination of the Degtjareff methods for determining soil organic matter and carbon in soils. Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Sci.* **37**:29-37.

How to Cite this Article:

Bhuvaneshwari K and Ajay Kumar. Agronomic potential of the association *Azolla –Anabaena*. *Sci. Res. Rept.,* **3**(1):78-82.