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Role of chemical characters of the pond water growing *Azolla* and the anthocyanin pigment present inside dorsal cavity of the fern in population growth in two kind of ponds of Jabalpur district of Madhya Pradesh

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Abstract

A study was conducted at J.N.K.V.V. Jabalpur to find out which of the chemical character of the pond water growing azolla, ammonia, nitrate, sulphur, phosphate and iron govern the population growth in two kinds of of ponds? Several studies have been performed on the effect of phosphorus fertilization on growth and N_2fixation activity of the *Azolla-Anabaena* symbiosis. These observations indicate that a P level which produces good growth may not be sufficient for maximum N accumulation. Also, Watanabe (1987) reported that 6 split applications totalling 15 kg P_20_5 ha^{-1}resulted in equivalent growth and N accumulation by *Azolla* . What role is attributable to the anthoyanins pigments normally present inside dorsal leaf of the fern endowing it the charterstic red colour in nature or in green house? Long standing and stabilized ponds having water for at least 11 months throughout a year were selected for this study. One site of Barhatatal had a record of partial coverage by azolla pinnata and second site of had a record of full coverage. In laboratory conditions of green house ventilated net covered model water and p- deficient vertisol of village Surai were used in Leonard jars having a surface area of 0.019 m2 .Control of deionised water was employed and 7 levels of applied phosphate (0,5, 10,30 60 and 120 ppm) were added to the soil lots . Two sets were organised , one of diffused light by using a cover of transparent polythene film and two of shaded light using a cover of blue polythene film. Our finding indicated that only phosphate controlled population density at the partial coverage pond and further, the sulphate and iron control it but nitrate negatively at full coverage pond. Anthocinin pigment is responsible for redness of frond and it is irreversible reaction means red never become green in entire life cycle of azolla.

Keywords: chemical character, populations growth, anthocyanin pigment deionzed water, P- Deficient vertisol.

1. Introduction

Rice is the single most important source of food for people and *Azolla* plays a very important role in rice production. For centuries *Azolla* and its nitrogen-fixing partner, *Anabaena*, have been used as "green manure" in China and other Asian countries to fertilize rice paddies and increase production. Some authorities believe the use of *Azolla* enabled the Vietnamese to survive the effects of the American blockade when imported fertilizers did not reach North Vietnam during the war. According to Wilson Clark (1980), the People's Republic of China has 3.2 million acres of rice paddies planted with *Azolla*. This provides at least 100,000 tons of nitrogen fertilizer per year worth more than $50 million annually. Extensive propagation research is being conducted in China to produce new varieties of *Azolla* that will flourish under different climatic and seasonal conditions. According to some reports, *Azolla* can increase rice yields as much as 158 percent per year. Rice can be grown year after year,

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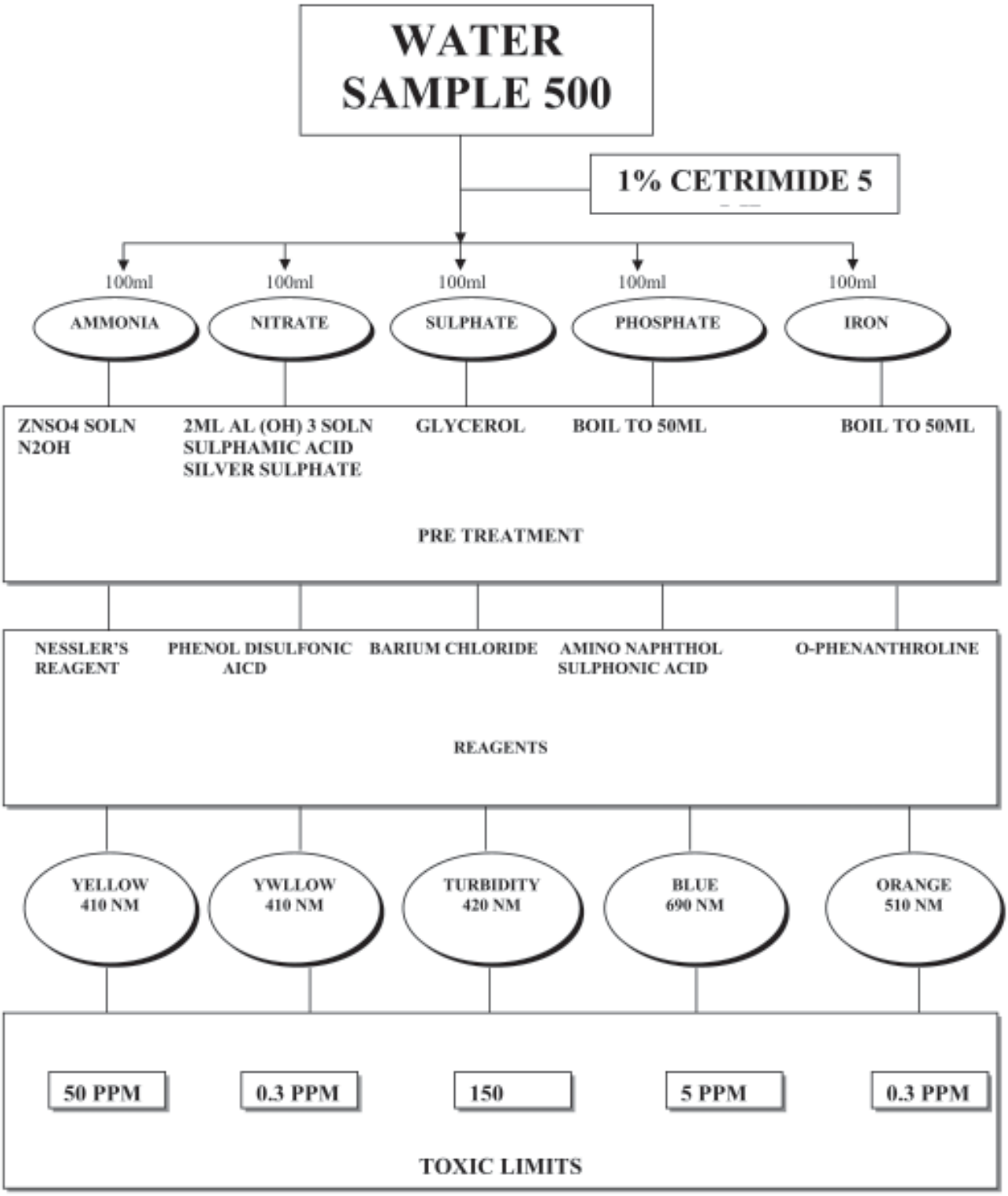
several crops a year, with little or no decline in productivity; hence no rotation of crops is necessary. *Azolla* var. pinnata is most probably the first hydrophytes likely to be born on the oldest crust of our earth, present in or around the city. It is a unique example of obligate symbiosis incorporating N2 fixing cynobacterium, Anabaena azollae. The importance of azolla in low land cultivation has been evaluated in numerous investigations(Liu, X.2008). Two kinds of status of pond were selected one, under partial coverage and two under full coverage by the fern. *Azolla* is grown either alongwith rice or singularly is turned down into the soil to supplement the nitrogen requirement of rice . In order to evaluate the influence of shade, created by tillering of rice having azolla sp. As dual or inter crop, a Green House experiment was set up in deionised water and in one P- deficient Vertisol using several applied P- levels (0-120ppm) Shading effect was simulated by the use of a blue film cover to allow a part of radiant energy (475-500nm) Effect of both the constraints, solar energy and the applied phosphate levels appeared mainly upon chlorophylls' and partially upon biomass.

2. Materials and methods

Jabalpur is located at 23.10 North Latitude at 79.59 East Longitude and is 411.78M above MSL. The area experience a tropical monsoonal climate . It has maximum rainfall in August –September . In the winter months, the SE monsoon is weakly active and thus, there is sporadic rainfall with slight drizzle during March and April .For field studies at site I ,which is partially covered by azolla has been intensively selected as it was studied under AICRP on Biological Nitrogen fixation (Verma and Dube 1984) for provenance trial, ecological distribution and thermotolerance of Azolla. The sampling of water was done for 9 months from September and ending in May. Two dates 5 and 20 every month were fixed but due margin of +_2 days keeping in view of various factor of weather and approachability. Sampling dates because of weather condition that are shown by numerals signifying 01 rain, 02 cool, 03 worm, 04hot, 05 placid, 07 cloudy. The random number was assigned to each of the duplicate sample under azolla and under water bodies. However site – II is another pond situated on Jhansighat Road, 45 km away towards west from Jabalpur city. This site was selected for one specific reason that azolla grew uncontaminated by lemna sp and spreading throughout the water body with no clear patches this giving a appearance of carpet. The sampling of this site was also done from September onwards up to May for 09 months in duplicate for one set of conditions i.e. under azolla only for describing the weather condition similar numerals have been used as similar to site 1 . The collection method followed were from Manual of Limnology (1986) .For sampling rubber stopped 500 ml capacity empty glucose saline bottle was brought in a inverted conditions under water bodies its rubber stopper removed and than open to fill in the water sample. When completely filled it was stoppered after adding 5 ml of 1% centrimide . For sampling water under azolla the inverted bottles was immersed at a given point and then brought under floating azolla where the frond was completely undisturbed and placid. At site II , azolla frond grew unlike at site I uniformly throughout the water body . Hence water body sample was out of consideration and only "under azolla "sample was available . Two replications of the sample were taken at two points at either site. All samples were brought to the laboratory and stored in a refrigerator until analyzed.

3. Analysis of water sample

At site I being the vicinity of the village Barha, had a source of Organic waste material available in the form of cattle dung human faces and off wash from both. On the other hand site II, being away from human habitat was relatively less polluted from organic waste. Both site had pH 6.5 and thus there is no possibility of interference of heavy metals except Organic waste to be encountered during chemical analysis of water samples. Spectrophoto-meter systronic model 118 was used to read the absorbance of the sample or the standard at respective length as shown in the flow diagram, water sample were analysed for ammonia, nitrate, sulphate, phosphate and iron by the procedure laid down in the Manual Standard Methods of Examination of Water and Waste water (AWWA-1976) sample collected under section 3.2 were also subject to analysis as per flow diagram me (fig-1)



Flow diagrams of sample of water at selected sites for chemical analysis of ammonia, nitrate, sulphate and Iron. A laboratory experiment was formulated to evaluate the influence of phosphate levels and the effects if shade due to tillering of rice in rice fields upon biomass and chlorophyll content of *Azolla* .Net house experiments was also designed for the treatments 0 ppm to 120 ppm phosphorus in two condition first is diffused vertisol of village Surai that had been studied under the ACIAR – ICAR project. Eighty Leonard jars were placed in east west direction so that maximum sunlight was available during day time and solar energy be utilized to a maximum levels by the growing azolla.Treatments

of the phosphate and set of diffused and shaded conditions and 5 intervals constituted the 3 factors involved in the design one column of eight treatment was sacrificed at the end of each of the 5 intervals and observation was recorded.When growing in full sunlight, particularly in late summer and onwards, *Azolla* may produce reddish anthocyanin in the leaves, in contrast with the bright green carpets of duckweed and filamentous green algae was studied(fig 2).

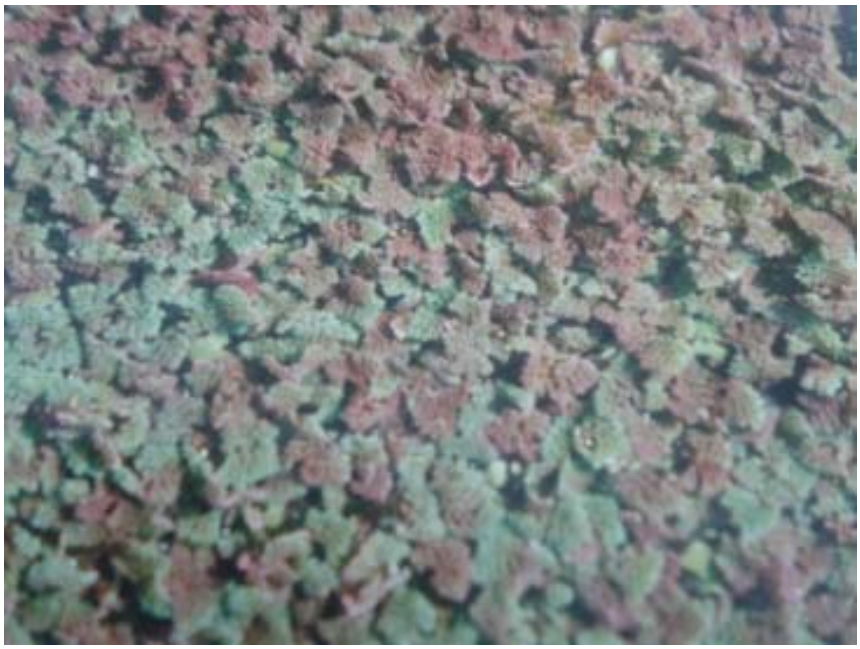


Fig. 1 : Redness of *Azolla pinnata* is due to Anthocyanin pigment

4. Results and discussion

Toxicity of water weather on two dates of sampling at two sites, found that the water at Barhatal (partially covered) achieved the toxic level for nitrate in January and February for sulphate in December towards and for iron throughout the experiment period water at site II Thoon total achivers toxic level for nitrate in November and onwards for sulphate in Octomer and onwards and for iron throughout the experiment priod. At site –I Barhatal on first date of sampling correlation between population density and other determinates showed a strong possible correlation between density and ammonia, ammonia leakage, sulphate and phosphate but a non significant with iron.

A significantly possible association of ammonia leakage with ammonia was quite axiomatic that nitrate recorded possible correlation with sulphate and phosphate was noteworthy. Out of six determinants for *azolla* population density only three (1) ammonia, (2) ammonia leakage & (3) phosphate achieved the level of significance. It will be seen that ammonia leakage had lost significance on the second date of sampling. The probable reason seems to be weather condition prevailing on the second date of sampling that is gale was present and the water had been turbulence. Further more it is from the graph that phosphate levels achieved maximum in January the population ammonia & ammonia leakage had their maximum in April. However at site II of Thoontatal (full covered) pond on the first date of sampling correlation between azolla population density and other determinants showed storong and positive association between azolla population and ammonia, nitrate sulphate and phosphate there was a negative correlation with iron but it was non significant.

Path analysis reveled that phosphate had a highest direct possible effect. (0.4496)followed by ammonia (0.346) sulphate (0.1977) and nitrate (o.034) Iron had the least direct negative effect (0.003) .Similar results were obtained for the first date and second date. Out of the five determinants for population density only three,. (1) Ammonia (2) Sulphate (3)Phosphate achieved the levels of significance. It will be noted that none of the determinants turned non significant on the second date of sampling as it happens in the case of site I. The obvious reasons being equivalence of weather condition under laboratory study from ANOVA table of biomass it becomes clear that both treatment and set were significant but not their

interaction . Among the various treatment of phosphate 10ppm prove to be superior to all other treatment which with the exception of 120ppm were on per to that level. The highest dose did not promote the biomass, shedding recorded an adverse effect and did not promote the biomass equally.

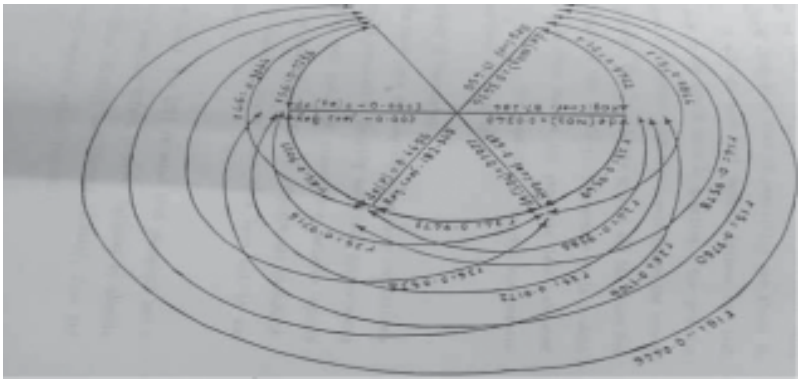


Fig. 2 : Path analysis of correlation between *Azolla* and other determinants

5. Conclusion

In conclusion, only phosphate controlled population density at the partial coverage site I, Barhatal and further the sulphate, phosphate and iron controlled it but nitrate negatively at full coverage site II of Thootatal (Full covered)Whereas the population and ammonia reached their maximum two months later than the phosphate could reach its maximum at the partial coverage both the two factor and phosphate reached their maximum synchronously at the full coverage. The later site was further characterized by a liner rise of sulphate uninterrupted by decline in population which subscribed to the existence of a dynamic sulphur pool in that limnic state as well.

In conclusion, the finding that *azolla* in control having deionized water alone grew very well it was assumed that the transplant material must have provide a least amount of phosphate as observed by the rise of general vigour 19 DAT onwards and when it was attacked by some fungi. Effect of Shedding permitting radiant energy of 470 -500 nm increased the population decreased the biomass and influenced the chlorophylls. A similar effect of shedding was equally observed with the p-deficient soil culture. The finding that 10 ppm phosphate was optimum for population and biomass had set the limit of critical levels. Its salutary influence only on chlorophyll is noteworthy. Besides this level, 60 ppm phosphate proved promotive also in this factor. Beyond 30ppm phosphate over duration showed a declining trend in population and biomass but on increasing trend in chlorophylls similar to the shedding effect. However our study for anthocinin pigment found that the red colour is due to presence of the pigment .It was found as irreversible reaction because once it change the colour of *azolla* as red it never become green in the entire life cycle of *azolla*.

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