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IMPACT OF PHYSICO-CHEMICAL PROPERTIES OF GROUND WATER AND SOIL ON NUTRITIVE VALUE OF *AZOLLA PINNATA* R. BR.

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ABSTRACT

This study was conducted at PG and Research Centre in Biotechnology, M.G.R. College, Hosur, Krishnagiri District, Tamilnadu, India during the year 2014 to evaluate the effect of physico-chemical properties of soil and water on nutritive value of *Azolla pinnata* R.Br. Ground water and soil samples were collected from same site of two districts i.e. Krishnagiri and Villupuram, India in winter season. The physico-chemical analysis results reported that Krishnagiri samples possessed more suitable physico-chemical properties with pH 7.25, Mg 4417.8, Ca 1978.3 and K 76.5 mg kg^{-1} for soil and 7.9, 187.3, 564.6 and 61.6 mg/L^{-1}, respectively for water in case of azolla cultivation than Villupuram district samples. These difference were directly reflected on nutritive value of *A. pinnata* R.Br. as the culture grown on Krishnagiri samples contained higher amount of nutritive value (crude protein 23.21%, crude vitamins 21.7%, crude fibre 15.9%, crude amino acid 7.9%, phosphorus 0.23%, magnesium 0.49%) than on Villupuram samples (crude protein 22.17%, crude vitamins 19.6%, crude fibre 13.3%, crude amino acid 7.4%, phosphorus 0.18%, magnesium 0.41%) except nitrogen free extract (36.12%) and potassium (3.6%). It is concluded that physico-chemical properties of Krishanagiri soil samples effecively supported the growth and nutritive value of azolla.

KEYWORDS: *Azolla pinnata*; nutritive value; soil; water; chemicophysical properties; trace elements; biomolecules; influence; India.

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INTRODUCTION

The water and soils are an indispensable natural resources on earth and are essential to all forms of life. Among these water makes up 50-97% of the weight of all plants and animals. The fertile soil and fresh polluted free water is absolutely essential for healthy living (Alalade *et al.,* 2007). Groundwater, suitable fresh water resources and fertile soils are providing all the sufficient needs for plants that include terrestrial and water fern plant ((Hassan *et al.,* 2012). The physico-chemical properties of soil and water are very essential for healthy vegetation and these resources determine the nutritional quality of plants especially water fern (Cherryl *et al.,* 2014; Ramesh and Vannila, 2012). Recently, the azolla a water fern has emerged as one of effective supplemented feeds for cattle and poultry animals. This is an aquatic plant and free floating fern which belongs to the family of *Azollaceae* and order Pteridophyta. There are six species of azolla widely used as feed for cattle and poultry which is commonly found in tropics and sub-tropics (Pillai *et al.,* 2005). It grows naturally in stagnant water of drains, canals, ponds, rivers, and marshy lands. *Anabaena-azollae*, living in the cavity of azolla leaf, can fix high amount of atmospheric dinitrogen due to presence of symbolic algae in the leaves (Basak *et al.,* 2002 and Pillai *et al.,* 2005).

Azolla is a good source of protein and contains almost all essential amino acids, minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese, etc, apart from appreciable quantities of vitamin A, precursor beta-carotene and vitamin B12 (Kumar *et al.,* 2012). Hence, azolla appears to be a potential source of nutrients and has a considerably high feeding value. *Azolla pinnata* is used as feed in broiler chicken (Balaji *et al.,* 2009), laying hens (Alalade *et al.,* 2007), juvenile black tiger shrimp (Sudaryono, 2006) and goats and buffalo calves (Indira *et al.,* 2009). With the above considerations, aim of present study was to analyse the impact of physico-chemical properties of ground water and soil on nutrient contents of *Azolla pinnata* R.Br.

MATERIALS AND METHODS

Source of water and soil sample and *Azolla pinnata* culture

This study was conducted at PG and Research Centre in Biotechnology, M.G.R. College, Hosur, Krishnagiri District, Tamilnadu, India during the year 2014.

The groundwater and soil samples were collected randomly from same sites of two district of Tamilnadu viz. Villupuram and Krishnagiri during the winter season. These samples were called as Krishnagiri and Villupuram samples. The *Azolla pinnata* culture was obtained from National Institute of Animal Nutrition and Physiology (NIANP), Adugodi, Bangalore, Karnataka state.

Physico-chemical properties analysis of samples

The groundwater and soil samples were collected in a separate clean plastic container and immediately transferred to laboratory for analysis of physical and chemical characters. The physical parameters like temperature, EC and pH were measured. Chemical analyses were carried out for the major, minor and trace ions. Further total dissolved solids, dissolved oxygen, total hardness and total alkalinity were analysed, using standard procedures of American Public Health Association (AOAC, 2007). All the reagents used were analytical grade (EMerk Pvt. Ltd., India and HiMedia Lab.Pvt.Ltd., India). The concentrations of heavy metals were calculated by modified method of Gitimoni Deka and Krishna Bhattacharyya (Rashid *et al.,* 2014). These water and soil samples were used for *Azolla pinnata* cultivation.

***Azolla pinnata* cultivation on silpaulin sheets (Azolla pond liner)**

The *Azolla pinnata* was cultivated in three pits each having the dimensions 5 ft x 10 ft with 1ft depth. Leveling of pit was properly checked to maintain a uniform water level and removed the roots and other unwanted particles in soil (Cherryl *et al.,* 2014). Silpaulin sheets (Azolla pond liner) of 7 ft X 12 ft longer and broader than pits, were spread out over the pits, with high water proof. Sheets were spread out uniformly and fixed properly. A layer of 5 cm clean soil (without dust and large stones) was spread evenly over and water was filled to a three fourth level in each pit and regular care was taken to maintain the same level of water. About 10 g of super phosphate dissolved in 10 liters of water was added to the soil in a zigzag manner (without cow dung). Once the preparation was successfully completed, each pit was inoculated with 2 kg of fresh and pure culture of *Azolla pinnata* and water was sprinkled over it. Once the culture was inoculated, it was covered with mosquito net to avoid culture contamination by pests and diseases. The culture and pit conditions were regularly monitored.

Harvesting and storage of *Azolla pinnata*

Within 10-15 days of inoculation the *Azolla pinnata* R. Br. culture was multiplied effectively. Fully grown culture was harvested every week regularly from the pits. Harvested culture was washed thoroughly in clean water, weighed and shadow dried for 2 to 3 days till it became crispy while green colour still retained in the dried *Azolla pinnata*. Dried *Azolla pinnata* was collected, packed in air tight bags and stored in aluminium feed bins until further use (Cherryl *et al.,* 2014).

Nutritional evaluation of *Azolla pinnata*

The dried samples were analyzed for proximate principles viz. dry matter, crude protein, ether extract, total ash, crude fibre, nitrogen free extracts, etc. and minerals such as potassium, calcium, phosphorus, magnesium, etc. were analysed as per methods described by AOAC (2007).

RESULTS AND DISCUSSION

Physico-chemical properties of soil and water

The pH and most of other parameters of water and soil used for *Azolla pinnata* cultivation fell within the range of permissible limit of Bureau of Indian Standard (IS-10500:1991). The total physico-chemical properties of soil and water are given in Table 1 and 2. The existing physical parameters of both district samples (soil and water) had most suitable range for *Azolla pinnata* (pH 7.25 and 7.29 in Krishnagiri, 6.8 and 6.6 in Villupuram samples) cultivation whereas temperature ranged 21-33ºC. Noticeably, the optimum temperature for *Azolla* spp. is between 18ºC and 28ºC, although some species can survive a very wide temperature range of about -5ºC to 35ºC (Gregory and Wagner, 1997). The trace elements play very important role in determining the healthy growth and nutrient content of *Azolla pinnata* (Basak *et al.,* 2002).

The phosphate in soil was 6.3 and 6.0 kg/acre for Villupuram and Krishnagiri samples, respectively Potassium found was 89.3 and 76.5 kg/acre in soil and 31.6 and 61.6 mg/l in water for Villupuram and Krishnagiri samples, respectively (Table 1 and 2). These act as basic elements to *Azolla pinnata.* Calcium content is very common in soil and groundwater, because it is abundantly derived from most of the rocks due to its higher solubility nature. However, range of its availability to plants like water ferns depends on the solubility of calcium carbonate and sulphate (Das, 2013; Rashid *et al.,* 2014). The calcium content was 1978.3 and 2256.8 mg/kg in soil and 564.6 and 318.6 mg/l in water for Krishnagiri and Villupuram sample, respectively. The calcium, magnesium and total hardness in the groundwater are inter-related. Similar results were reported by Ramesh and Vennila (2012). The overall physico-chemical property reports of ground water and soil samples

Table 1. Physico-chemical properties of soil used for *Azolla pinnata* R. Br. cultivation

Properties	Villupuram	Krishnagiri	Permissible limit
pH	6.8	7.25	6-8
Temperature	30ºC	33ºC	-
EC (dSm^{-1})	1	1	0.1-1
$CaCl_2$	Nil	Nil	-
Texture	RLL	RLL	-
N (kg/acre)	*92	*98	114-180
P (kg/acre)	6.3	6	4.6-9
K (kg/acre)	89.3	76.5	49-113
Ca^{2+} (mg/kg^1)	2256.8	1978.3	52000
Mg^{2+}	3245.7	4417.8	9000
Cd^{+}	2.5	3.8	2-6
Cu^{+}	78.3	91.6	100
Fe^{+}	1924.8	2017.5	129000
Zn^{+}	498.1	596.1	300-600
Cr^{-}	69.3	76.3	1000
Mn^{+}	527.9	613.2	1000
Pb^{-}	107.8	175.8	200

Table 2. Physico-chemical properties of ground water used for Azolla pinnata R. Br. cultivation.

Properties	Villupu-ram	Krishn-agiri	Permissible limit
pH	6.6	7.29	6.5-8.5
Temperature	21ºC	28ºC	-
TDS (mg/l)	3108.1	3412.4	500-2000
EC(µmhos/cm)	3156.3	327 1.1	700-3000
Total hardness (mg/l)	645.2	947.3	300-500
Ca^{2+} (mg/l)	318.6	564.6	75-200
Mg^{2+} (mg/l)	124.5	187.3	30-100
Na^{+} (mg/l)	364.6	481.2	200-300
K^{+} (mg/l)	31.6	61.6	10
$HCO3^{-}$(mg/l)	472.5	503.8	500
Cl^{-}(mg/l)	462.8	559.5	250-1000
NO_3^{-}(mg/l)	64.2	93.1	45
F^{-}(mg/l)	2.9	4.7	1.5
Fe^{-}(mg/l)	0.75	1.62	1.0
Pb^{-}(mg/l)	1.73	3.2	0.5
Cr^{-}(mg/l)	0.04	0.01-1.8	0.5

TDS: Total dissolved solids, **EC:** Electric conductivity

state that sample (soil and water) taken and used for *Azolla pinnata* R.Br. cultivation from Krishnagiri district possess least excess quantity of most trace elements as compared to samples of Villupuram district, which are essential for healthy growth of *Azolla pinnata*.

Physico-chemical impact of samples on nutritive value of *Azolla pinnata*.

Analysis of shadow dried *Azolla pinnata* samples revealed that dry matter contents of culture grown in Villupuram and Krishnagiri water and soil samples were almost similar i.e.89.13 and 87.15%, respectively (Table 3). These results are in close agreement with the results of Balaji et *al*. (2009) and slightly lower than value obtained by Kumar *et al.* (2012). The less dry matter content of *Azolla pinnata* may act as an impediment to use it on fresh basis as the bulk required to satisfy the dry matter requirements of livestock is very high (Niaz *et al.,* 2014; Cherryl *et al.,* 2014). The organic matter and crude protein contents of culture *Azolla pinnata* from Krishnagiri samples show more quantity (73.23 and 23.21%) than Villupuram samples (71.15 and 22.17%) (Table3). It indicated that almost high physico-chemical property of Krishnagiri sample is responsible for this differentiation which may provide more nourishment energy to increase the nutritive value of *Azolla pinnata* than other samples. This statement and estimated crude protein value was almost similar to the results obtained by Balaji *et al.* (2009) and Kumar *et al.* (2012). However, Basak *et al.* (2002) and Cherryl *et al.* (2014) reported higher value above 26.62%. This variation may be attributed to the physico-chemical property of water and soil used for *Azolla pinnata* cultivation.

Table 3. Nutrient contents variation in dry matter of *Azolla pinnata* R. Br. grown on two sites.

S. No	Nutrient (%)	Villupuram	Krishnagiri
1	Dry matter	89.13	87.15
2	Organic matter	71.15	73.23
3	Crude protein	22.17	23.21
4	Crude vitamins	19.6	21.7
5	Ether extract	2.9	3.9
6	Crude fibre	13.3	15.9
7	Nitrogen free extract	36.12	33.95
8	Total ash	21.19	26.27
9	Acid insoluble ash	6.17	8.35
10	Crude amino acid	7.4	7.9
11	Potassium	3.6	3.2
12	Calcium	1.0	1.02
13	Phosphorus	0.18	0.23
14	Magnesium	0.41	0.49

The data further indicate (Table 3) that crude fibre, crude vitamins and ether extracts were greater in Krishnagiri (15.9, 21.7, and 3.9%) samples than Villupuram samples (13.3, 19.6 and 2.9%) which were similar to the results of Balaji *et al.* (2009). The total ash content of *Azolla pinnata* obtained from Krishnagiri samples was 26.27%, which was higher than Villupuram sample (21.19%). It may be due to higher and sufficient amount of trace and NPK elements of water and soil samples of Krishnagiri. These results agree to the findings of Titus and Periera (2007) and Kumar *et al.* (2012). The nitrogen free extract content of *Azolla pinnata* recorded in this study was higher in Villupuram samples (36.12%), than Krishnagiri samples (33.95%) which were partially similar to the findings of Kumar *et al.*

(2012) and Bolka (2011). Present study exposed that calcium contents in dried *Azolla pinnata* were lower (1.0% in Villupuram and 1.02% in Krishnagiri samples) than reported by Cherryl *et al.* (2014) (2.58%). The K, P and Mg contents were less in both samples (Table 3), as observed by Cherryl *et al.* (2014).

CONCLUSION

The overall results conclude that physico-chemical properties of soil and water played important role in growth and nutrient contents determination of *A. pinnata* R.Br. Physico-chemical property of Krishnagiri samples effectively supported the growth and nutrient content of *A. pinnata* culture than Villupuram samples except nitrogen free extract and potassium. It may be due to lower trace element contents in soil and water than Krishnagiri samples and over-warm climatic conditions of the district.

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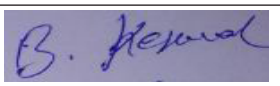
COUNTRIBUTION OF AUTHORS

S. No.	Name of author	Contribution	Signatures
1.	B. Kesuva	Collected sample	
2.	N. Mathiyazhagan	Prepared writeup	
3.	K. Suresh	Helped in Lab tests	
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