International Journal of Prevention and Control of Industrial Pollution
Vol. 1: Issue 1
www.journalspub.com

Elemental Attributes of the *Bacopa monnieri*

Shikha Suman*
Botany Department, Banaras Hindu University, Varanasi (Uttar Pradesh), India

Abstract
The heavy metal concentration in the water used for irrigation also deplete the plant cycle as it has the accumulation within the plant cells. The different heavy metals also alter the absorption of the nutrient elements from the water and then indirectly affect the plant growth and consequently reduces the plant yield, the assessment in this paper is regarding how the heavy metal accumulation will alter the plant physiology and finally the plant yield.

Keywords: *medicinal plants, heavy metal, accumulation, plant growth*

****Author for Correspondence:*** *Email ID: shikhasuman.bhu@gmail.com*

INTRODUCTION
The experiment was set with a medicinally and economically important plant *Bacopa monnieri*, a member of Scrofulareaceae family[1] used as a traditional medicine since ancient time[2], and due to over exploitation due to high demand as raw material in the Drug company put this plant under the category of the endangered, to save this highly important plant Tissue culture studies was made on this plant. To compare the micro-propagated and the vegetative propagated plant propagule, the plant was raised *invitro*[1] and in vivo and studied maximum plant functional traits under daily and alternate water stress[3,4]. As we found the Soil Water content plays important role in the life physiology of a wet land plant[3]. The propagated plants are named as MP (micro propagated) ad VP (vegetative propagated) with dual water stress implementation i.e., daily watering (MPD, VPD) and alternate watering (MPA, VPA). Thus four experimental sets were designed. To examine the effect and governess of the soil water content on the different plant morphological, physiological and biochemical content[5], we statistically define the interconnection, inter-relation, and dependence of the plant traits on the soil water content[6].

HEAVY METALS
Heavy metals and metalloids emitted from automobile, mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping and military operations make a significant contribution to environmental pollution as a result of human activities[7]. These pollutants present a risk for primary, secondary and top consumers[8]. In optimum quantities Mn and Zn are essential for plant growth and development[9] because they are constituents of many enzymes and other proteins. However, elevated concentrations of both essential and non-essential heavy metals in the soil can lead to toxicity and inhibits the plant growth[10,11]. Toxicity may result from the binding of metals to sulfhydryl groups in proteins, leading to inhibition of activity or disruption of structure, or from displacement of an essential element, resulting in deficiency effects[12]. Further, excess heavy metal stimulates the formation of free radicals and reactive oxygen species and may result in oxidative stress[10].The lifetime of active oxygen species within the cellular

environment is determined by the antioxidant system (Enzymes and compounds of low molecular weight) provides crucial protection against oxidative damage[11].

The antioxidant properties of plants exposed to various stress factors have been studied[13], but studies related to heavy metal-induced variations are lacking. Pb and Hg were reported to cause an increase in ascorbic acid levels in two *Oryza sativa* cultivars[14–16]. Reports suggested that heavy metals and metalloids have effects on chlorophyll and amino acid content in plants. Heavy metals are known to interfere with chlorophyll synthesis either through direct inhibition of an enzymatic step or by inducing deficiency of an essential nutrient[17]. Heavy metal accumulation in plants can affect plant productivity, food quality and human health[18]. The Pb accumulation in human blood via food chain has been reported[18] to cause cognitive development and reduce intellectual performance of children and result in a number of cardiovascular dysfunctions in adults[14–18]. Cadmium (Cd) can cause kidney damage, impair skeletal and reproductive systems and other health problems[15]. These heavy metals are used as bioaccumulaters in this study and also to decide the suitable condition for growth and development of the plant material used in this study.

NUTRIENTS

Qualitative and quantitative determination of mineral nutrients present in plant is important for human health. The quality of food depends on the quantity and type of minerals present therein[14]. The food rich in mineral nutrients serves a significant role against a variety of degenerative diseases, prevent or reduce injury from environmental pollutants and enhance the ability to work and learn. Due to this view point the quantification of the mineral nutrients (Ca, Fe, Mg, Na, K, Zn, Ni and Co) in plant material is important to study (The *Ayurvedic Pharmacopoeia* of India 1999). These inorganic elements play an important role in physiological process involved in human health[14]. K is important as diuretic and it takes part in ionic balance of the human body and maintains tissues excitability. It is the principal intracellular cation and also consider as a very important constituent of the extracellular fluids[14]. Potassium ions are concerned with the transmission of electrical impulse in the nerve cells and in maintaining the fluid balance of the body. Venkataraman and Gopal Krishnan[19, 20] reported maximum concentration of Ca, Fe and K in plants traditionally used for jaundice and concluded that high concentration of K in the medicinal plants could be related to the diuretic action of drugs prepared from these plants[21].Calcium (Ca) imparts strength and rigidity to bones and teeth. Ca ions are needed in neuromuscular transmission, in excitability of nerves for normal excitability of heart, in clotting of blood and promoting muscular contraction[22,23]. It also acts as an activator of the enzymes (i.e., phospholipase, argininekinase, adenosine triphosphatase and adenyl kinase) [24, 25]. Excess quantity of Ca ions in the extracellular fluids acts as a mental depressant, at the other extreme, low level of Ca causes spontaneous discharge of nerve fibers, resulting in tetany[23]. Magnesium (Mg) is the fourth most abundant cation in the body. Much of Mg is present in bones in association with Ca and Phosphate and the rest in soft tissues and body fluids. In muscles and other tissues, intracellular Mg ions function as activators for many of the enzymes involved in carbohydrate metabolism and synthesis of DNA and RNA. It also acts as binding agent of ribosomal particles where protein synthesis takes place[21]. Increased extracellular concentration of Mg depresses skeletal muscle contraction[25]. On the other hand, low Mg concentration causes enhanced irritability in humans and also affects the nervous system, peripheral vasodilation and cardia arrhythmias[22]. Manganese (Mn) is essential for haemoglobin formation but excess is harmful. Zink (Zn) is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, alkaline phosphatase, carbonic anhydrase and procarboxypeptidase[26]. Zn is crucial for the normal growth and reproduction and helps in the process of tissue repair and wound healing[27]. Zn insufficiency causes growth retardation and skin lesions[28]. The lesser amount of Zn accumulation in the plants is due to its less absorption from the soil[21]. Phosphate ions are the major anions of intracellular fluids, phospholipids and the coenzyme NAD and NADP and especially of ATP and other high energy compounds. It helps in the process of ossification of bones by getting deposited in the form of Calcium Phosphate. Nickle (Ni) aids the synthesis of haemoglobin in the bone marrow[21]. Iron (Fe) performs a wide range of biological functions; many of these functions are connected with oxidation-reduction processes and thus results in energy accumulation in the body. It forms an integral part of cytochromes, haemoglobin, myoglobin, metallo flavoproteins and certain enzymes such as catalase and peroxidises[25–28].

HEAVY METALS PRESENCE IN *BACOPA* PLANT

The heavy metals present in *Bacopa monnieri* across the age, mode of propagation and watering condition are shown in Table 1 a and b. Across the age, mode of propagation and watering condition the value (µg g^{-1}) of Cu, Cd, Cr, Ni, Pb, Zn, Fe and Mn varied from 54 to 101, 8.10 to 28.22, 23 to 66, 14 to 72, 11 to 40, 51 to 142, 840 to 2583 and 25 to 63, respectively, as shown in Table 1 a and b. The value of Cd, Cr, Ni and Pb were minimum in MPD and maximum in VPA, opposite to this the value of Fe was minimum in VPA and maximum in MPD. On the other hand, the value of Zn and Mn were minimum in VPD and Maximum in MPA. The Cu was also minimum in VPD but maximum in VPA as shown in Table 6. In addition, the value of Cu, Cr, Zn and Fe decreased consistently due to plant age as depicted in Figure 1. The patterns of Cd and Pb due to plant age were concave while for Ni and Mn was convex represented in Figure 1.

Table 1a: *Heavy Metals (µg g^{-1}) in Micro propagated Bacopa Monnieri under Daily and Alternate Watering Condition.*

Heavy Metals	Daily			Alternate		
	A	B	C	A	B	C
Cu	87.07	77.03	74.20	91.43	87.83	70.67
	(1.89)	(5.26)	(3.94)	(5.54)	(3.25)	(1.91)
Cd	15.00	8.10	14.80	20.01	15.31	18.70
	(3.60)	(1.11)	(2.31)	(1.30)	(2.72)	(3.11)
Cr	35.67	36.23	23.47	45.67	38.87	26.00
	(3.13)	(0.91)	(1.9)	(3.68)	(2.91)	(2.72)
Ni	14.27	26.33	22.00	25.67	49.77	38.50
	(1.05)	(5.6)	(3.18)	(4.29)	(1.36)	(1.15)
Pb	14.33	10.67	13.23	23.30	13.27	24.47
	(0.81)	(1.36)	(2.05)	(2.01)	(1.76)	(4.39)
Zn	122.43	88.63	70.90	142.03	90.83	82.50
	(19.3)	(3.07)	(8.27)	(18.96)	(3.82)	(7.36)
Fe	2583	1818	1264	2052	1527	853
	(92.1)	(105)	(15)	(108)	(54.9)	(29.69)
Mn	45.00	52.93	33.37	54.37	62.93	36.53
	(3.18)	(1.42)	(2.51)	(4.57)	(2.12)	(2.87)

A, B, and C are 120, 240 and 360 days after transplantation, respectively. The values in the parentheses are ±SD.

Table 1b: *Heavy Metals (µg g^{-1}) in Vegetative Propagated Bacopa Monnieri under Daily and Alternate Watering Condition.*

Heavy Metals	**Daily**			**Alternate**		
	A	**B**	**C**	**A**	**B**	**C**
Cu	74.17	66.80	53.50	100.70	88.57	78.27
	(2.81)	(1.59)	(2.4)	(7.39)	(3.33)	(5.1)
Cd	23.62	14.73	24.60	28.22	25.32	25.41
	(1. 51)	(1.50)	(3.02)	(1.81)	(1.30)	(4.11)
Cr	53.97	50.10	34.77	65.77	46.70	43.00
	(1.95)	(1.83)	(3.71)	(3.05)	(6.05)	(2.2)
Ni	35.30	53.30	47.50	43.63	71.80	56.23
	(1.15)	(2.91)	(2.61)	(2.78)	(3.22)	(2.6)
Pb	30.03	22.10	29.70	39.80	26.40	36.07
	(3.1)	(2.95)	(1.45)	(3.4)	(1.9)	(4.8)
Zn	80.43	64.90	51.00	124.47	87.53	65.23
	(5.71)	(3.4)	(1.28)	(16.4)	(2.21)	(3.3)
Fe	1919	1509	1047	1377	1245	840
	(86.16)	(41.53)	(20.59)	(46.66)	(35.85)	(24.2)
Mn	36.73	25.67	25.40	29.27	35.37	32.43
	(3.72)	(2.55)	(3.72)	(3.51)	(4.06)	(4.05)

A, B, and C are 120, 240 and 360 days after transplantation, respectively. The values in the parentheses are ±SD.

The Repeated Measure analysis of variance showed significant effects of propagation mode, watering condition on all the heavy metals. Age also exhibited notable differences in the values of heavy metals due to age, except for Cd, Pb and Fe. Further, the analysis suggested that the Fe and Mn varied statistically due to two way and three way interactions of age, propagation and watering condition (Table 2).

Table 2: *Summary of Repeated Measure ANOVA on the Heavy Metals of Bacopa Monnieri due to Propagation Mode, Watering Condition and Age of the Plant Material. $^{ns}P > 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.*

Parameter	**Age (A)** $F_{(1,8)}$	**Propagation (P)** $F_{(1,8)}$	**Watering (W)** $F_{(1,8)}$	**A×P** $F_{(1,8)}$	**W×P** $F_{(1,8)}$	**A×W** $F_{(1,8)}$	**A×P×W** $F_{(1,8)}$
Cu	209.8***	6.17*	64.41***	3.19^{ns}	3.33^{ns}	33.88**	1.34^{ns}
Cd	0.34^{ns}	206.3***	84.94***	0.009^{ns}	0.75^{ns}	0.00^{ns}	0.23^{ns}
Cr	275.01***	209.72***	27.13*	5.14*	6.14*	0.05^{ns}	0.76^{ns}
Ni	81.44***	368.29***	161.49***	0.70^{ns}	1.19^{ns}	5.31*	0.87^{ns}
Pb	0.62^{ns}	278.67***	72.37***	0.66^{ns}	0.05^{ns}	0.21^{ns}	1.25^{ns}
Zn	203.7***	26.84**	22.89**	2.56^{ns}	7.29*	3.94*	2.42^{ns}
Fe	1.30^{ns}	478.86***	518.78***	103.8***	17.43**	4.95*	3.84*
Mn	45.91***	721.76***	72.6***	14.70*	2.23^{ns}	12.63**	13.89**

Tukey HSD test revealed that the values of Cu, Cr, Fe and Ni differed significantly amongst the age groups. The values of Cd and Pb were also different between 120 and 240 DATs and between 240 and 360 DATs. The values of Mn were significantly different between 120 and 360 DATs and between 240 and 360 DATs as shown in Figure 1.

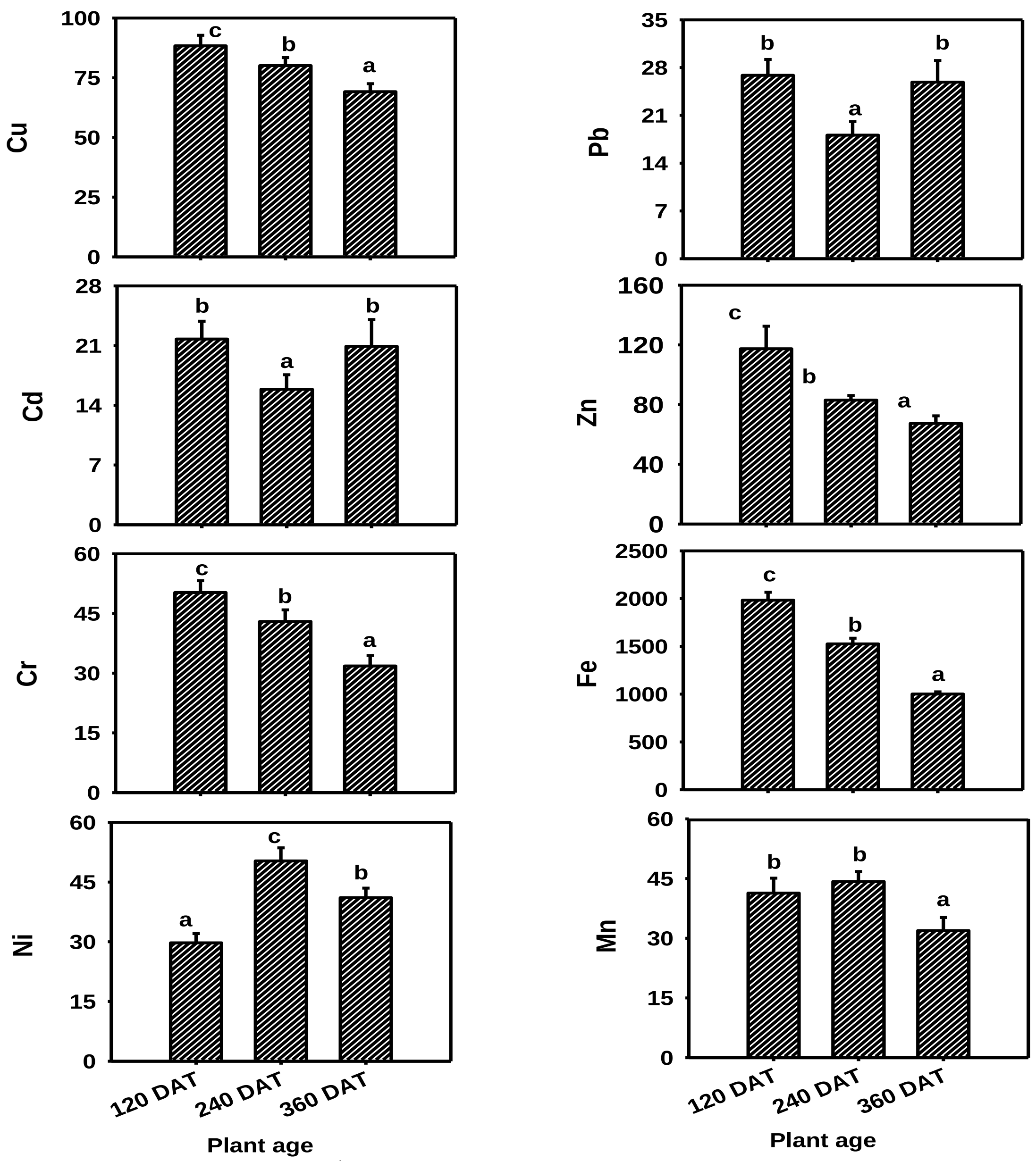

Fig 1: *Heavy Metals (µg g^{-1}) Variation in Bacopa Monnieri due to Plant Age. Different Letters within a Diagram are Significantly Different at P ≤0.05.*

NUTRIENT ELEMENTS PRESENCE IN *BACOPA* PLANT

The nutrient elements present in *Bacopa monnieri* under different experimental treatments are included in Table 3. This table showed that the quantitative values (in µg g^{-1} for Mg, Na, Ca, K, and P and in % for N and C) of these elements across the age, mode of propagation and watering condition differed from 662–1892, 519–1237, 1003–2466, 3454–9076, 0.46–3.57, 0.18–2.04, 26.07–41.17 and 14–196, respectively for Mg, Na, Ca, K, P, N, C and C : N ratio (Table 3). The values of Mg, Ca, K, P, and N were minimum in VPA and maximum in MPD. In contrast to this, the values of Na, C and C:N ratio were minimum in MPD and maximum in VPA (Table 3). These elements showed differential response to plant age. For example; the Mg, P and N had convex shape, while C and C: N ratio had concave. The values Na and Ca increased consistently while K decreased due to increased plant age as shown in Table 3 a and b.

Table 3a: *Nutrient Elements (μg g^{-1}) in Micropropagated of Bacopa Monnieri under Daily and Alternate Watering Condition.*

Parameters	Daily			Alternate		
	A	B	C	A	B	C
Mg	1499	1892	1293	1226	1460	963
	(24.01)	(34.33)	(18.6)	(62.23)	(20.59)	(55.16)
Na	519	665	721	651	915	1037
	(8.64)	(7.77)	(52.21)	(15.68)	(14.89)	(73.86)
Ca	1763	2460	2466	1287	1910	1921
	(188.7)	(244.6)	(300.3)	(139.0)	(41.97)	(54.96)
K	8415	9076	4438	8129	8162	3454
	(335.5)	(63.37)	(216.5)	(117.1)	(106.8)	(97.0)
P	1.64	3.57	1.15	1.29	2.00	0.85
	(0.09)	(0.39)	(0.11)	(0.04)	(0.12)	(0.05)
N	0.57	2.04	0.47	0.55	1.00	0.35
	(0.06)	(0.1)	(0.06)	(0.03)	(0.04)	(0.03)
C	27.95	27.59	29.04	29.45	27.40	29.99
	(0.47)	(1.71)	(0.72)	(0.52)	(0.59)	(1.29)
C : N ratio	49	14	62	54	27	86
	(7.35)	(1.87)	(9.21)	(8.78)	(2.13)	(8.84)

A, B, and C are 120, 240 and 360 days after transplantation, respectively. The values of N and C are in percent. The values in the parentheses are ±SD.

Table 3b: *Nutrient Elements (μg g^{-1}) in Vegetative Propagated of Bacopa Monnieri under Daily and Alternate Watering Condition.*

Elements	Daily			Alternate		
	A	B	C	A	B	C
Mg	1124	1486	745	1040	1125	662
	(20.13)	(16.26)	(10.13)	(18.72)	(41.84)	(29.1)
Na	820	1009	1069	977	1149	1237
	(23.08)	(3.98)	(44.56)	(10.65)	(75.32)	(31.73)
Ca	1528	1606	1990	1009	1111	1003
	(78.77)	(271.5)	(17.46)	(6.55)	(2.53)	(2.06)
K	6153	7413	6135	5489	4438	4866
	(105.9)	(116.1)	(88.01)	(205.9)	(216.5)	(124.3)
P	1.05	1.86	0.76	0.89	1.27	0.46
	(0.08)	(0.14)	(0.02)	(0.07)	(0.03)	(0.04)
N	0.44	0.82	0.31	0.36	0.55	0.18
	(0.02)	(0.01)	(0.02)	(0.03)	(0.03)	(0.03)
C	29.24	26.07	30.60	40.17	32.82	35.26
	(0.57)	(0.39)	(1.59)	(0.45)	(1.85)	(0.45)
C :N ratio	66	32	99	112	60	196
	(8.52)	(4.15)	(18.31)	(25.14)	(7.25)	(29.19)

A, B, and C are 120, 240 and 360 days after transplantation, respectively. The values of N and C are in percent. The values in the parentheses are ±SD.

The Repeated Measure suggested significant effects of propagation mode, watering condition on all the nutrient elements. Age too, showed remarkable distinctions in the values of nutrient elements due to age, except for Mg and K. Further, the revealed that the plant carbon content differed statistically due to two ways and three way interactions of age, propagation and watering condition (Table 4).

Table 4: *Summary of Repeated Measure ANOVA on the Nutrient Elements of Bacopa Monnieri due to Propagation Mode, Watering Condition and Age of the Plant Material.* $^{ns}P > 0.05$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Nutrients	Age (A) $F_{(1,8)}$	Propagation (P) $F_{(1,8)}$	Watering (W) $F_{(1,8)}$	A×P $F_{(1,8)}$	W×P $F_{(1,8)}$	A×W $F_{(1,8)}$	A×P×W $F_{(1,8)}$
Mg	1.28^{ns}	634.22***	335.67***	70.82**	2.82^{ns}	35.26**	2.88^{ns}
Na	440.03***	327.4***	143.54***	2.3^{ns}	13.91**	5.75*	10.8*
Ca	38.31**	135.74***	136.56***	9.26*	3.43^{ns}	1.97^{ns}	1.9^{ns}
K	1.55^{ns}	433.2***	422.79***	1.15^{ns}	30.61**	62.41***	0.15^{ns}
P	186.18***	245.6***	149.79***	2.88^{ns}	0.5^{ns}	19.16**	2.52^{ns}
N	62.85***	6600.77***	307.22***	0.01^{ns}	3.34^{ns}	53.29***	0.61^{ns}
C	4.48*	90.08***	105.65***	33.29**	57.69***	70.23***	40.68***

Tukey Honestly Significant Difference test indicated that the mean values of Mg, Na, P and C:N ratio between various age groups differed notably. The mean values of; Ca between 120 and 240 DATS, between 120 and 360 DATs, K between 120 and 360 DATs, between 240 and 360 DATs and C between 120 and 240 DATs and between 240 and 360 DATs varied significantly as shown in Figure 2.

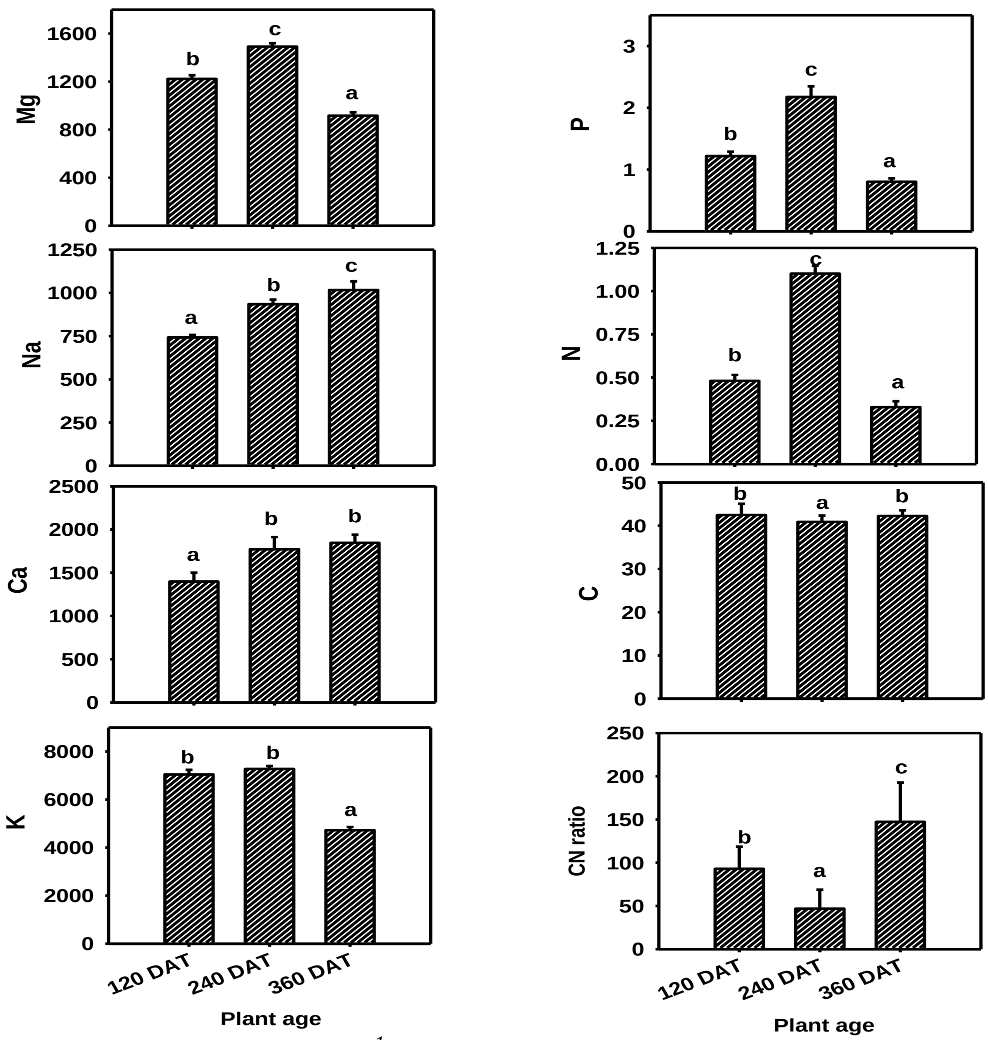

Fig. 2: *Nutrient Elements (µg g^{-1}) Variation in Bacopa Monnieri due to Plant Age. The Values of N and C are in Percent. Different Letters within a Diagram are Significantly Different at $P \leq 0.05$.*

Across the age, propagation mode and watering condition, correlation coefficients among various nutrient elements of *Bacopa monnieri* are presented in Table 4. Results suggested that among the studied nutrients Mg and C: N ratio was correlated with maximum numbers of element variables. The C: N ratio was negatively correlated with Mg, K, P and N and positively with Na and C. The Mg was also related with those nutrient variables which were related with C: N ratio, but the nature of relationship was opposite (Table 5).

Table 5: *Pearson Correlation (r) Matrix among Different Nutrient Elements of Bacopa Monnieri. Values in the Parentheses are Significance Level (P) at n = 12.*

Parameters	Mg	Na	Ca	K	P	N	C
Na	-0.69						
	(0.01)						
K	0.71	-0.63	0.23				
	(0.01)	(0.03)	(0.48)				
P	0.92	-0.51	0.53	0.72			
	(0.00)	(0.09)	(0.08)	(0.01)			
N	0.85	-0.42	0.53	0.66	0.98		
	(0.00)	(0.18)	(0.08)	(0.02)	(0.00)		
C	-0.61	0.48	-0.66	-0.51	-0.55	-0.48	
	(0.04)	(0.11)	(0.02)	(0.09)	(0.07)	(0.12)	
CN	-0.86	(0.61)	-0.54	-0.59	-0.75	-0.67	0.73
	(0.00)	(0.04)	(0.07)	(0.04)	(0.00)	(0.02)	(0.01)

HEAVY METALS AND NUTRIENT ELEMENTS COMPARATIVE STUDY

Heavy metals inhibit physiological processes such as respiration, photosynthesis, cell elongation, plant-water relationship, N-metabolism and mineral nutrition[20]. Metals can be transported *via* an apoplastic system and immobilized in cell walls[22]. Toxic metals become a real threat to plants mainly when they reach to the cytosol of the cell. Therefore, the ability of root cells to control the transport of heavy metals *via* membranes determines tolerance by plants[25]. They can be immediately complexed, inactivated and transformed into a physiologically tolerable form *via* action of phytochelatins and sequestred in cell vacuoles[28]. Ni accumulation damages the cellular parts of the leaves, alters its water metabolism, pigment and reserve material synthesis and finally inhibits the yield production[22,18]. Decreased Fe concentration has been associated with reduction of chlorophyll content. Reduction in Fe concentration is also associated with decrease in the activities of the Fe enzymes, catalase and peroxidase, and thus, reduced availability of Fe for chlorophyll–heme biosynthesis[21].

Mg being a part of chlorophyll compound plays a fundamental role in photosynthesis and phloem export of photosynthates from the source to the sink organs, and its deficiency in plants results in dramatic reduction of photosynthesis and translocation of carbohydrates from leaves to other plant organs[26]. Thus, the study suggested that in 240 DAT the presence of larger amount of Mg in the studied plant could promote the accumulation of biomass and bacoside contents. The negative relationship between Mg and Na also confer for the greater accumulation of biomass and bacoside contents in middle age of the plant. The accumulation of Na is primary cause of the ion specific damage[15] and protein synthesis[18]. It has been reported that Na ion compete with Ca ion for binding sites under stress condition, and Ca accumulation in plants ameliorates the negative effects of drought stress on plant growth and development[25]. Further, positive relationship of Mg with K, P and

N also strengthen the above statement. As, K is involved in a large number of essential processes for plant growth, including enzyme activation, protein synthesis, photosynthesis, regulation of osmotic pressure, vascular transport, and cation-anion balance[28]. P affects the photosynthetic rate per unit area and its deficiency reduces the leaf growth and photosynthetic rate may be attributed to reduction in stomatal conductance and ribulose 1, 5 bisphosphate (RuBP) and carboxylase regeneration capacity[22], Also, it is required for processes including the storage and transfer of energy, photosynthesis, the enzyme regulation, and the transport of carbohydrates[28]. The positive effects of P on plant growth have been attributed to an increase in stomatal conductance[27], photosynthesis[28], higher cell-membrane stability, water relations[25] and drought tolerance. Phosphorus improves the root growth and maintains high leaf water potential.

The heavy metals also inhibit the activity of the enzymes by interaction with -SH groups[26] and changing the cationic balance at sub cellular level[22]. The accumulation of these heavy metals inhibit the ATPase activity, consequently reduce the active absorption of the minerals[18]. The increased activity of the peroxidase and hydrolases induces the dark respiration due to the accumulation of these metals and therefore causes premature leaf senescence[14–20]. Also, the increased ethylene production[21] causes the leaf senescence[22–25]. These physiological changes due to heavy metal accumulation in *B. monnieri* resulted into the morphological changes (Table 6).

Table 6: *Pearson Correlation Coefficients (r) of Different Heavy Metals with Different Traits of Morphological and Physiological Functional Groups and with Bacoside Contents of Bacopa Monnieri Values in parentheses are significance levels (P) at n = 12.*

Traits	**Cu**	**Cd**	**Cr**	**Ni**	**Pb**	**Zn**	**Fe**	**Mn**
Shoot length	-0.08	-0.68	-0.31	-0.10	-0.74	-0.18	0.16	0.28
	(0.65)	(0.00)	(0.06)	(0.56)	(0.00)	(0.31)	(0.35)	(0.10)
Root length	0.40	-0.22	0.29	-0.38	-0.36	0.52	0.85	0.49
	(0.02)	0.19	(0.08)	(0.02)	(0.03)	(0.00)	(0.00)	(0.00)
Plant fresh weight	0.79	-0.26	0.98	0.78	-0.98	0.76	0.81	0.45
	(0.00)	0.13	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.01)
Plant dry weight	0.63	-0.16	0.76	0.95	-0.74	0.96	0.90	0.55
	(0.00)	0.34	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Moisture content	-0.27	-0.40	-0.11	-0.50	-0.43	0.01	0.44	-0.01
	(0.11)	(0.02)	0.51	(0.00)	(0.01)	0.96	(0.01)	0.94
Leaf area	0.29	-0.41	0.11	0.27	-0.46	0.15	0.20	0.53
	(0.08)	(0.01)	(0.54)	(0.11)	(0.00)	(0.40)	(0.24)	(0.00)
Biomass	-0.23	-0.52	-0.21	0.20	-0.51	-0.29	0.00	0.35
	(0.17)	(0.00)	(0.22)	(0.25)	(0.00)	(0.09)	(0.99)	(0.04)
RS ratio	0.44	0.60	0.66	0.65	0.55	0.07	-0.22	-0.09
	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.68)	(0.20)	(0.58)
NPP	0.41	-0.40	0.38	-(0.06)	-0.42	0.40	0.67	0.49
	(0.01)	(0.02)	(0.02)	(0.72)	(0.01)	(0.01)	(0.00)	(0.00)
RGR	0.01	-0.60	0.07	-0.31	-0.46	0.09	0.52	0.39
	(0.94)	(0.00)	(0.69)	(0.06)	(0.01)	(0.59)	(0.00)	(0.02)
SLA	0.12	-0.34	0.12	-0.40	-0.45	0.34	0.70	0.28
	(0.50)	(0.04)	(0.47)	(0.02)	(0.01)	(0.04)	(0.00)	(0.09)
SLW	-0.07	0.33	-0.05	0.34	0.43	-0.31	-0.55	-0.23
	(0.70)	(0.05)	(0.76)	(0.04)	(0.01)	(0.07)	(0.00)	(0.19)
LAR	0.52	-0.14	0.44	-0.27	-0.12	0.71	0.60	0.18
	(0.00)	(0.42)	(0.01)	(0.11)	(0.50)	(0.00)	(0.00)	(0.29)
NAR	0.04	-0.61	0.06	-0.30	-0.47	0.09	0.51	0.42

	(0.83)	(0.00)	(0.72)	(0.08)	(0.00)	(0.59)	(0.00)	(0.01)
Ash content	-0.39	-0.73	-0.49	-0.34	-0.77	-0.16	0.40	0.37
	(0.02)	(0.00)	(0.00)	(0.04)	(0.00)	(0.36)	(0.02)	(0.03)
Ion leakage	0.34	0.72	0.53	-0.02	0.77	0.32	-0.11	-0.31
	(0.04)	(0.00)	(0.00)	(0.92)	(0.00)	(0.06)	(0.54)	(0.06)
Evapotranspirational	0.31	0.72	0.66	0.17	0.78	0.31	-0.04	-0.18
Water loss	(0.07)	(0.00)	(0.00)	(0.32)	(0.00)	(0.07)	(0.81)	(0.28)
WUE	-0.21	-0.62	-0.26	0.09	-0.59	-0.27	0.04	0.35
	(0.22)	(0.00)	(0.12)	(0.59)	(0.00)	(0.11)	(0.83)	(0.04)
RWC	-0.13	-0.80	-0.34	-0.67	-0.72	0.18	0.57	0.33
	(0.46)	(0.00)	(0.05)	(0.00)	(0.00)	(0.29)	(0.00)	(0.05)
pH	-0.42	-0.62	-0.08	-0.02	-0.48	-0.38	0.17	0.00
	(0.01)	(0.00)	(0.63)	(0.90)	(0.00)	(0.02)	(0.32)	(0.98)
Total chlorophyll	0.04	-0.79	-0.24	-0.47	-0.81	0.25	0.62	0.71
	(0.80)	(0.00)	(0.15)	(0.00)	(0.00)	(0.15)	(0.00)	(0.00)
Total carotenoid	0.16	-0.40	-0.26	-0.42	-0.46	0.49	0.38	0.44
	(0.35)	(0.02)	(0.12)	(0.01)	(0.00)	(0.00)	(0.02)	(0.01)
Crude extract	0.22	-0.65	-0.02	-0.29	-0.70	0.30	0.61	0.69
	(0.20)	(0.00)	(0.91)	(0.08)	(0.00)	(0.07)	(0.00)	(0.00)
Bacoside	0.34	-0.54	0.12	-0.35	-0.60	0.46	0.71	0.61
	(0.04)	(0.00)	(0.48)	(0.04)	(0.00)	(0.00)	(0.00)	(0.00)
Bacoside *a*	0.09	-0.77	-0.15	-0.47	-0.79	0.27	0.69	0.63
	(0.61)	(0.00)	(0.37)	(0.00)	(0.00)	(0.11)	(0.00)	(0.00)
Reducing sugar	0.46	-0.44	0.01	-0.02	-0.53	0.40	0.38	0.85
	(0.00)	(0.01)	(0.97)	(0.93)	(0.00)	(0.02)	(0.02)	(0.00)
Starch	0.26	-0.54	-0.11	-0.65	- 0.61	0.51	0.90	0.47
	(0.13)	(0.00)	(0.51)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Soluble sugar	0.62	-0.37	(0.05)	-(0.09)	-0.50	0.51	0.41	0.85
	(0.00)	(0.03)	(0.79)	(0.62)	(0.00)	(0.00)	(0.01)	(0.00)
Sucrose	-0.03	0.04	-0.50	-0.41	0.00	0.15	-0.24	0.00
	(0.87)	(0.81)	(0.00)	(0.01)	(0.98)	(0.38)	(0.17)	(0.98)
Phenol	0.37	0.77	0.39	0.25	0.74	0.33	-0.27	-0.10
	(0.03)	(0.00)	(0.02)	(0.14)	(0.00)	(0.05)	(0.11)	(0.55)
Protein	0.19	-0.78	-0.16	-0.27	-0.80	0.22	0.56	0.71
	(0.28)	(0.00)	(0.34)	(0.12)	(0.00)	(0.21)	(0.00)	(0.00)
Proline	0.35	0.02	-0.07	-0.50	-0.03	0.59	0.27	0.40
	(0.04)	(0.92)	(0.68)	(0.00)	(0.87)	(0.00)	(0.11)	(0.01)

Minerals play a major role in determining the growth of the plant[22]. In present study, the N, P, and K contents were significantly related with the growth promoting traits[33] as shown in Table 7. Plants respond to a gradual increase in availability of mobile nutrient first by increasing their vacuolar reserves[22], in second stage, accumulate in the tissues of leaves and stems[24] and then the photosynthetic[25] and growth rates[26] rises. Mg increases chlorophyll formation, ATP formation in chloroplasts, CO_2 fixation, protein synthesis, phloem loading, partitioning and utilization of photo-assimilates, generation of reactive oxygen species, and photo-oxidation in leaf tissues[28]. In the present study, the accumulation of the Na in the plant generates the salt-induced-leaf growth reduction[14], results in decreased WUE[20] by increasing evapotranspirational water loss[21], inhibits the root growth and hence the capacity of the water uptake and ultimately lowers the nutrient absorption[28]. The accelerated loss of leaf area due to Na accumulation leads to leaf senescence[28] and thus reduced photosynthesis[27], decreased plant biomass and finally reduced the plant productivity[28]. It also maintains the essential hormones to growth limiting level and further growth is hampered as depicted in Table 7. The Na accumulation interacts with the K and Ca absorption. It has been reported that reduction in the sugar quantity under salt stress condition may be attributed to suppress the amylase activity[20] which causes reduced hydrolysis of reserve polysaccharides. Na accumulation exerts its effect through membrane peroxidation suggesting that formation of oxygen free radicals during stress increases the phenol accumulation in plants. The decrease in the protein content could be related to less photosynthesis and plant growth[29].

Table 7: *Pearson Correlation Coefficients (r) of Different Nutrient Elements with Different Traits of Morphological and Physiological Functional Groups and with Bacoside Contents of Bacopa Monnieri Values in parentheses are significance levels (P) at n = 12.*

Traits	Mg	Na	Ca	K	P	N	C	C/N
Shoot length	0.77	-0.31	0.59	0.33	0.72	0.70	-0.59	-0.73
	(0.00)	(0.07)	(0.00)	(0.05)	(0.00)	(0.00)	(0.00)	(0.00)
Root length	0.58	-0.64	(0.03)	0.65	0.43	0.32	-0.39	-0.58
	(0.00)	(0.00)	(0.88)	(0.00)	(0.01)	(0.05)	(0.02)	(0.00)
Plant fresh weight	0.79	-0.20	0.44	0.56	0.90	0.92	-0.48	-0.71
	(0.00)	(0.24)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Plant dry weight	0.53	0.08	0.13	0.39	0.62	0.64	-0.29	-0.51
	(0.00)	(0.66)	(0.44)	(0.02)	(0.00)	(0.00)	(0.09)	(0.00)
Moisture content	0.52	-0.55	0.57	0.30	0.42	0.38	-0.50	-0.62
	(0.00)	(0.00)	(0.00)	(0.08)	(0.01)	(0.02)	(0.00)	(0.00)
Leaf area	0.67	-0.08	0.10	0.47	0.71	0.70	-0.30	-0.69
	(0.00)	(0.65)	(0.58)	(0.00)	(0.00)	(0.00)	(0.07)	(0.00)
Biomass	0.56	0.00	0.41	0.48	0.74	0.78	-0.47	-0.52
	(0.00)	(0.98)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
RS ratio	-0.27	0.54	-0.69	-0.12	-0.19	-0.16	0.61	0.29
	(0.11)	(0.00)	(0.00)	(0.49)	(0.28)	(0.34)	(0.00)	(0.09)
NPP	0.81	-0.44	(0.02)	0.71	0.76	0.71	-0.33	-0.78
	(0.00)	(0.01)	(0.91)	(0.00)	(0.00)	(0.00)	(0.05)	(0.00)
RGR	0.77	-0.48	0.36	0.65	0.87	0.87	-0.42	-0.56
	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)
SLA	0.65	-0.66	0.34	0.52	0.49	0.40	-0.44	-0.84
	(0.00)	(0.00)	(0.04)	(0.00)	(0.00)	(0.02)	(0.01)	(0.00)
SLW	-0.58	0.57	-0.37	-0.39	-0.43	-0.35	0.41	0.84
	(0.00)	(0.00)	(0.02)	(0.02)	(0.01)	(0.03)	(0.01)	(0.00)
LAR	0.49	-0.49	-0.11	0.33	0.30	0.23	-(0.07)	-0.57
	(0.00)	(0.00)	(0.54)	(0.05)	(0.07)	(0.18)	(0.68)	(0.00)
NAR	0.78	-0.47	0.36	0.64	0.87	0.88	-0.40	-0.56
	(0.00)	(0.00)	(0.03)	(0.00)	(0.00)	(0.00)	(0.01)	(0.00)
Ash content	0.59	-0.46	0.62	0.50	0.54	0.46	-1.00	-0.64
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Ion leakage	-0.62	0.16	-0.57	-0.37	-0.68	-0.65	0.75	0.58
	(0.00)	(0.35)	(0.00)	(0.03)	(0.00)	(0.00)	(0.00)	(0.00)
Evapotranspirational	-0.57	0.24	-0.74	-0.22	-0.54	-0.52	0.69	0.54
Water loss	(0.00)	(0.16)	(0.00)	(0.19)	(0.00)	(0.00)	(0.00)	(0.00)
WUE	0.64	-0.08	0.51	0.49	0.78	0.82	-0.52	- 0.58
	(0.00)	(0.64)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
RWC	0.77	-0.74	0.61	0.66	0.66	0.59	-0.66	-0.65
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
pH	0.64	-0.15	0.49	0.44	0.67	0.65	-0.61	-0.59
	(0.00)	(0.37)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)
Total chlorophyll	0.86	-0.66	0.56	0.77	0.80	0.74	-0.78	-0.82
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Total Carotenoid	0.39	-0.50	0.24	0.27	0.22	0.13	-0.49	-0.57

	(0.02)	(0.00)	(0.16)	(0.11)	(0.20)	(0.44)	(0.00)	(0.00)
Crude extract	0.88	-0.57	0.44	0.74	0.85	0.79	-0.60	-0.88
	(0.00)	(0.00)	(0.01)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Bacoside	0.83	-0.63	0.29	0.67	0.71	0.64	-0.52	-0.87
	(0.00)	(0.00)	(0.08)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Bacoside *a*	0.64	-0.29	0.14	0.59	0.68	0.67	-0.34	-0.62
	(0.00)	(0.08)	(0.41)	(0.00)	(0.00)	(0.00)	(0.04)	(0.00)
Reducing sugar	0.70	-0.83	0.27	0.70	0.52	0.39	-0.53	-0.59
	(0.00)	(0.00)	(0.11)	(0.00)	(0.00)	(0.02)	(0.00)	(0.00)
Starch	0.62	-0.38	0.09	0.52	0.62	0.61	-0.16	-0.56
	(0.00)	(0.02)	(0.61)	(0.00)	(0.00)	(0.00)	(0.35)	(0.00)
Soluble sugar	-0.33	-0.11	0.18	-0.50	-0.42	-0.41	0.07	0.28
	(0.05)	(0.53)	(0.30)	(0.00)	(0.01)	(0.01)	(0.68)	(0.10)
Sucrose	-0.75	0.38	-0.71	-0.40	-0.72	-0.68	0.71	0.65
	(0.00)	(0.02)	(0.00)	(0.02)	(0.00)	(0.00)	(0.00)	(0.00)
Phenol	0.91	-0.53	0.47	0.75	0.89	0.83	-0.65	-0.81
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Protein	0.91	-0.53	0.47	0.75	0.89	0.83	-0.65	-0.81
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
Proline	-0.04	-0.39	-0.03	0.01	-0.18	-0.21	0.02	-0.04
	(0.83)	(0.02)	(0.88)	(0.97)	(0.29)	(0.21)	(0.93)	(0.81)

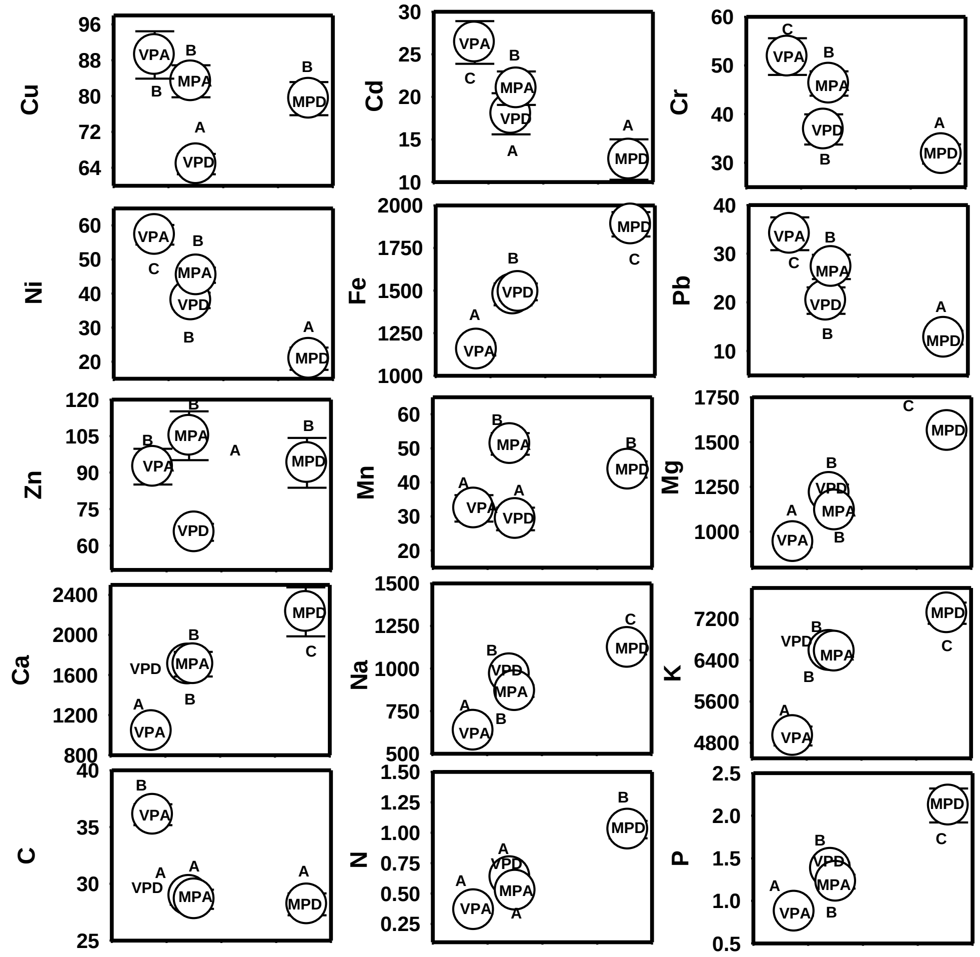

Fig. 3: *Elemental traits across the four experimental sets of the B. monnieri. Where the Cu, Cd, CR, Pb, Zn, Mn, Ni, Mg, Ca, Na, K and P were expressed in µg g-1, while, C and N were expressed in percentage.*

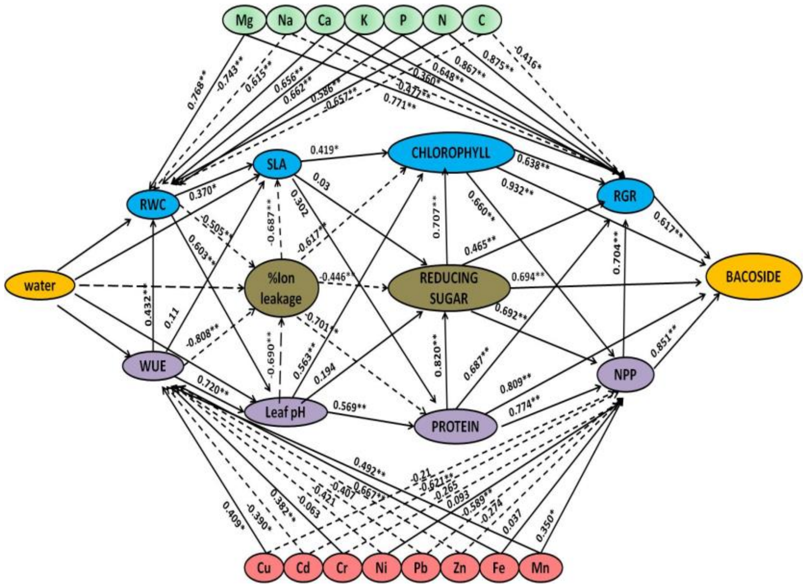

Fig. 4: *The hypothetical Blue ring of the proposed path model (Figure 3) showed the effect of the water content on the active yield of the bacoside content of the Bacopa plant via morpho-physiological processes while the hypothetical Purple ring indicates the effect of water on the bacoside via internal physiological factors. The Brown ring showed the path dependency on the stress factors. The green ring indicates the effect of the nutrient element on the most governing morpho-physiological factors (Relative water content (RWC) and Relative growth rate) while the red ring were the heavy metal, which were seems to highly affect the water use efficiency (WUE) and Net primary productivity (NPP) of the plant. The values on the lines are R at n = 12. $^{ns}P < 0.05$; $*P < 0.05$; $**P < 0.01$; $***P < 0.01$. The solid lines represent the positive significant relations while the dotted lines represent the negative relations.*

CONCLUSION

According to Figure 4 The relative water content (RWC) was inversely related to Dry matter content[29] while dry matter was directly proportional to Leaf carbon content (C)[30] which in turn was inversely proportion to SLA[31]. The SLA was directly related to NAR[32]. The leaf carbon content was strongly related to plant nutrient[33]. SLA had strong relation with plant nutrient concentration, WUE, average evapotranspirational loss, and RGR[34]. There was a close relationship between chlorophyll concentration and leaf Nitrogen content (N)[35]. According to some study[36-40], Chlorophyll was linked to leaf N and hence to growth rate (RGR). Chlorophyll was also a direct indicator of the plant productivity (NPP)[38]. Leaf N and P determined the growth rate (RGR), transpiration loss (AVET), SLA and NAR[39]. Leaf N and P scaled positively with one another and showed similar relationship with growth rate (RGR)[38]. Moreover SLA, Leaf C and N were tightly related to RGR[40]. According to Schulze *et al.*[34], evaporational loss is linearly related to Leaf N content. The leaf N is directly related to the protein content as it is the structural part of the protein[40]. The protein and the reducing sugars had been considered as stress relevant osmolytes which protected plants from the water stress[41]. The increased in the sugar and the osmolytes concentration enhanced the accumulation of the bacoside and crude content in the *B. monnieri* plant[42]. The heavy metal accumulation had the growth retarding property[43]. It also reduced the WUE of the plant and finally reduced the NPP[44]. Compartmentalization of the heavy metal in the cell organelle could reduce the negative impact of heavy metals on the growth and cellular metabolic activity of the *B. monnieri*[45]. Some scientist advocated that molecules in the cytoplasm like phytochelatins form complexes with the metals and facilitate their transport over the tonoplast into the vacuole and hence do not allow the heavy metal to cause any negative impact on the plants[45].

Important Factors Influencing the Variation in Crude Extract and Bacoside Content

There is web like interconnections amongst the morphological, physiological, biochemical and elemental traits of the *B. monnieri*. But the question is to find out the real traits which govern the production of crude extract and bacoside content in the studied plant material. For this; the step wise multiple regression was accomplished. Total models fitted for different functional groups by stepwise regression to control the variations in crude content and bacoside production in *B. monnieri* and the correlation coefficients of initial and final models are reported in Table 8. Further, the regression coefficients of the step wise equations of crude extract, bacoside contents with fitted traits of different functional groups as well as with fitted traits based on the traits of all functional groups are reported in Tables 9 and 10.

The analyses revealed that among different morphological traits; the leaf area alone governed the 72 % variability in crude extract, while along with root length, stem dry weight, root dry weight and percent plant moisture it governed 92% variability. For bacoside content; 56% difference was explained by the leaf area alone, along with the root length the total explanation in bacoside content was 74.5 % as depicted in Table 8. From the physiological traits; NAR controlled 74 % variation in crude extract, whereas, NAR along with, SLW, RWR, percent ash content, RS ratio and leaf pH explained 91 % variation. Instead of NAR, NPP governed 73% variation in bacoside content and in association with RS ratio the NPP explained 82% difference in bacoside content. Amongst the bio-compounds; it was the protein content which governed 88 % variability in crude extract, on the other hand, when it was combined with the ratio of chlorophyll *a* to *b*, soluble sugar, and the total explanation in crude extract variability was 94%. The Chlorophyll *a* explained 72 % variability in bacoside content. The chlorophyll *a* jointly with protein content explained 79% % variability. When the data was subjected to heavy metals; 48 % variation in crude extract was exhibited by the lead content and along with Mn and Cr the total explanation in variability in crude extract was 79 %. Among the heavy metals; Fe inhibited the bacoside production up to 51% while along with the accumulation of Pb and Cr in plant body the total explanation in variability was 76%.From the nutrients; Mg alone explained 78 % variation in crude extract and together with K the total explanation in variability was 80 %. Interestingly it was only Mg which explained 69 % variation in bacoside content in the *B. monnieri* as shown in Table 5.

When the overall traits were considered to explain the variation in crude and bacoside production, the results suggested that the protein and crude extract explained 93 and 99% variation in crude extract and bacoside production, respectively (Table 8-11). The protein along with SLA, reducing sugar, NAR, chlorophyll *a*: *b* and Pb explained 99 % difference in crude extract. However, the crude extracts along with LAR, bacoside *a*, P and NPP determine 96 % difference in bacoside production. Therefore, among the studied traits, bacoside production is dependent on the crude production, LAR, bacoside *a*, P and NPP. Nevertheless, the importance of SLA, NAR, reducing sugars and chlorophyll contents could not be ignored.

Table 8: *Total models fitted for different functional groups by stepwise regression to control the crude content (upper panel) and bacoside production (lower panel) in B. monnieri. Here only the correlation coefficients (r) of initial (r_1) and (r_2) are given.*

Functional groups	Models	r_1	±1SE	r_2	±1SE
Morphological parameters	5	0.85	0.07	0.97	0.03
Physiological parameters	6	0.85	0.07	0.96	0.03
Bio-compounds	5	0.93	0.05	0.97	0.03
Heavy metal	3	0.69	0.11	0.89	0.06
Nutrients	2	0.81	0.06	0.89	0.65
Pooling of all traits	6	0.93	0.05	0.99	0.02
Morphological parameters	2	0.75	0.73	0.86	0.56
Physiological parameters	2	0.85	0.58	0.91	0.47
Bio-compounds	2	0.85	0.56	0.88	0.51
Heavy metal	3	0.71	0.77	0.87	0.56
Nutrients	1	0.83	0.69	0.00	0.00
Pooling of all traits	5	0.90	0.47	0.96	0.32

Based on the above analyses, the study revealed that SLA, LAR, NAR, NPP, chlorophyll and P and Pb contents which were generally associated with plant photosynthesis were the exclusive parameters to govern the amount of production in *Bacopa minnieri*. It has been reported that greater amount of chlorophyll synthesis promotes the photosynthesis which determines the plant growth and yield[41]. In a study, the positive role of P on photosynthesis was also reported[37]. The higher concentrations of Pb inhibits the yield content of the plant[41, 39] by damaging the bio membranes and altering the enzymatic activities[37], thus reducing the photosynthesis. Therefore, the bacoside content in the studied plant is largely photosynthesis dependent.

Table 9: *Regression derived coefficients of stepwise multiple regression to predict the traits of different functional groups which influenced the crude extract of B. monnieri. Here the coefficients of only final model are included.*

Plant functional groups	Parameters	Slope (B)	±1SE	Beta	*t*	*P*
Morphological	(Constant)	0.05	0.04	0.00	1.52	0.14
	Leaf area	0.01	0.00	0.43	4.34	0.00
	Root length	0.02	0.00	0.30	5.45	0.00
	Shoot dry weight	0.02	0.00	0.67	8.02	0.00
	Root dry weight	-0.02	0.01	-0.35	-3.79	0.01
	Percent moisture content	0.00	0.01	0.20	2.93	0.01
Physiological	(Constant)	0.53	0.14	0.00	3.85	0.01
	NAR	0.04	0.00	0.67	9.13	0.00
	SLW	-0.25	0.04	-0.34	-5.55	0.00
	RWR	-0.63	0.17	-0.22	-3.66	0.01
	Percent ash content	0.01	0.00	0.37	4.48	0.00
	RS ratio	0.33	0.13	0.21	2.62	0.01
	Leaf pH	-0.06	0.03	-0.17	-2.14	0.04
Bio-compounds	(Constant)	0.12	0.02	0.00	5.09	0.00
	Protein	0.01	0.00	0.37	2.65	0.01
	Sol sugar	0.01	0.01	0.30	4.39	0.00
	Chlorophyll *a*	0.12	0.03	0.45	3.90	0.00
	Chlorophyll *b*	-0.10	0.03	-0.25	-3.00	0.01
	Chlorophyll *a :b*	-0.02	0.00	-0.32	-4.86	0.00
Heavy metal	(Constant)	0.31	0.08	0.00	4.04	0.00
	Pb	-0.01	0.00	-0.87	-6.75	0.00
	Cr	0.01	0.01	0.56	5.28	0.00
	Mn	0.00	0.01	0.28	2.62	0.01
Nutrients	(Constant)	- 0.08	0.04	0.00	- 1.81	0.08
	Mg	0.00	0.00	0.71	6.57	0.00
	K	0.00	0.00	0.24	2.20	0.04

Table 10: *Regression derived coefficients of stepwise multiple regression to predict the traits among different functional groups which influenced the bacoside content of B. monnieri. Here the coefficients of only final model are included.*

Category	Parameters	Slope (B)	±1SE	Beta	*t*	*p*
Morphological	(Constant)	0.64	0.27	0.00	2.40	0.02
	Leaf area	0.01	0.00	0.50	4.94	0.00
	Root length	0.20	0.04	0.49	4.85	0.00
physiological	(Constant)	3.56	0.21	0.00	16.72	0.00
	NPP	0.02	0.00	0.91	12.01	0.00
	RS ratio	-3.79	0.92	-0.31	-4.11	0.00
Bio-compounds	(Constant)	0.54	0.32	0.00	1.68	0.10
	Chlorophyll *a*	1.70	0.16	0.86	10.60	0.00
	Proline	0.71	0.26	0.22	2.75	0.01
Heavy metal	(Constant)	2.16	0.50	0.00	4.34	0.00
	Fe	0.01	0.00	0.24	2.00	0.05
	Pb	-0.10	0.02	-0.86	-5.76	0.00
	Cr	0.06	0.01	0.59	4.37	0.00
Nutrients	(Constant)	-0.37	0.39	0.00	-0.96	0.34
	Mg	0.00	0.00	0.83	8.78	0.00

Table 11: *Regression derived coefficients of stepwise multiple regression to predict the traits among all the studied traits which influenced the crude extract (upper panel) and bacoside production (lower panel) of B. monnieri. Here the coefficients of only final model are included.*

Parameters	B (Slope)	±1SE	Beta	*t*	*p*
(Constant)	-0.14	0.05	0.00	-2.93	0.01
Protein	0.02	0.00	0.64	5.92	0.00
SLA	0.00	0.00	0.24	5.30	0.00
Reducing sugar	0.01	0.00	0.22	3.62	0.00
NAR	0.04	0.02	0.11	2.45	0.02
Chlorophyll *a/b*	-0.01	0.00	-0.16	-3.46	0.00
Pb	0.00	0.00	0.17	2.59	0.02
(Constant)	0.01	0.41	0.00	0.02	0.98
Crude extract	4.12	1.10	0.54	3.74	0.00
LAR	0.02	0.01	0.18	2.61	0.01
Bacoside *a*	0.02	0.01	0.37	2.90	0.01
P Content	-0.40	0.14	-0.30	-2.81	0.01
NPP	0.01	0.00	0.24	2.26	0.03

Thus it was sorted by the help of multiple regression that in this study leaf area, protein content, chlorophyll content, Mg content were the some exclusive parameters which directly gave an account of the plant yield (crude content and bacoside content). The total canopy photosynthesis area (leaf area) was directly governed the yield of the plant[39]. The roots play an index role in determining yield[40]. The increased proline content retrieve the plant to recover from the stress[42] by aiding the chlorophyll synthesis and finally increase the plant yield[44]. Mg may directly or by enhancing the chlorophyll synthesis of plants, increases the plant yield[22]. The leaf phosphorus is the main nutrient element govern the leaf growth and finally the RGR, which is directly associated with the yield of the plant[26]. The sugars were the structural and non structural component of the plant alkaloid[37]. Cr is used as Hill reagent in the photosynthesis thus necessary for the plant growth and yield[39] but its higher concentration reduces the plant growth[40], Beside these the Cd and the Pb are the metals their accumulation decreases the plant yield[41,39] by damaging the bio membranes and altering the enzymatic activities[44].

REFERENCE

1. Suman S. Tissue Culture Studies on Bacopa monnieri: A Review. *Research and Reviews: Journal of Botany*. 2014; 3(3): 27–39p.
2. Suman S. Ethnobotany Emphasizing on Traditional Medicinal System: A Review. *Journal of Herbal Sciences*. 2014; 3(3): 51–4p.
3. Suman S. Water stress: An overview on plant life. *Journal of Ecology*. 2014; 3(3): 12–26p.
4. Suman S. Ecophysiological analysis of Micropropagated and vegetatively propagated Bacopa monnieri under daily and alternate water stress. *Ph.D. Thesis, Banaras Hindu University, Varanasi.* 2012.
5. Suman S., Kapur P., Sagar R. Phytodiversity potential: exploration through micropropagation. In: *Biotic Potential & the Abiotic Stress*. Dwivedi A.K., Srivastava M., Pandey V.N. Lamprecht Academy Publisher (LAP), Germany. 284–306p.
6. Suman S. Statistical Implementation of the Plant Functional Traits of Bacopa monnieri and Their Biological Interpretation, *Journal of Computational Biology*, 2014; 3(3): 13–23p.
7. Nedelkoska T.V., Doran P.M. Hyperaccumulation of cadmium by hairy roots of Thlaspi caerulescens. *Biotechnology and Bioengineering*. 2000; 67(5): 607–15p.
8. Herren T, Feller U.1997. Transport of cadmium via xylem and phloem in maturing wheat shoots: comparison with the translocation of zinc, strontium and rubidium. *Annals of Botany*. 80; 623–8p.
9. Marschner H., Cakmak I. 1989. High light enhances chlorosis and necrosis in leaves of zinc, potassium, and magnesium deficient bean (Phaseolus vulgaris) plants. *Journal of Plant Physiology*. 134: 308–15p.
10. Kabata-Pendias A., Pendias H. 1991. Trace Elements in Soils and Plants, 2ed. CRC Press.
11. Hall J.L. Cellular mechanisms for heavy metal detoxification and tolerance. *Journal of Experimental Botany*. 2002; 53: 1–11p.
12. Dietz K.J., Baier M., Krämer U. 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. In: Prasad MNV, Hagemeyer J, eds. Heavy metal stress in plants: from molecules to ecosystems. Berlin: *Springer-Verlag*. 73–97p.
13. Noctor G., Foyer C.H. Ascorbate and glutathione: keeping active oxygen under control. *Annu Rev Plant Physiol Plant Mol Biol*. 1998; 49: 249–79p.
14. Havaux M., Kloppstech K. 2001. The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in Arabidopsis npq and tt mutants. *Planta.* 213: 953–66p.
15. Mishra A., and Choudhuri M.A. Monitoring or phytotoxicity of lead and mercury from germination and early seedling growth induces two rice cultivars. Water, Air, and Soil Pollution. 1999; 114: 339 –46p.
16. Assche V., Clijsters H. Effect of metals on enzyme activity in plants. Plant Cell Environment. 1990; 13: 195–206p.
17. Alloway B.J. *Heavy metals in soils*. New York: John Wiley and Sons; 1990. ISBN 0470215984 EC (1999) Integrated Pollution Prevention and Control (IPPC) Best available techniques reference document on the production of iron and steel. Publ: EC European IPPC Bureau 370 p.
18. Jorgensen S.S. Mobility of metals in soils. *Folia Geogr Danica*. 1991; 104–14p.
19. Grath M., Zhao F.J., Lombi E. Plant and rhizosphere process involved in phytoremediation of metal-

contaminated soils. *Plant and Soil*. 2001; 232: 207–14p.
20. Venkataraman R., Gopalakrishnan S. Trace elemental profile in medicinal plants traditionally used for jaundice. *J Current Sci*. 2002; 2: 305–8p.
21. Vermani A., Navneet, Chauhan P.A. Physico-Chemical Analysis of Ash of Some Medicinal Plants Growing in Uttarakhand, India. *Nature and Sci*. 2010; 8(6): 88p.
22. Singh V., Jain D.K. *Nootan biology*. 7th Edn. Meerut, India: Nageen Prakashan Pvt Ltd; 2006.
23. Chatterjee M.N., Shinde R. *Text book of medical biochemistry*. 2nd Edn. New Delhi, India: Jaypee Brother Medical Pub Ltd; 1995; 811–46p.
24. Indrayan A.K., Sharma S., Durgapal D., *et al*. Determination of nutritive value and analysis of mineral elements for some medicinally valued plants from Uttaranchal. *Current Sci*. 2005; 89: 1252–5p.
25. Rusch G.M., Skarpe C., Halley D.J. Plant traits link hypothesis about resource-use and response to herbivory. *Basic Appl Ecol*. 2009; 10: 466–74p.
26. Pakeman R.J., Leps J., Kleyer M., *et al.*, and Vista Consortium. Relative climatic edaphic and management controls of plant functional traits signatures. *Journal of Vegetation Science*. 2009; 20: 95–102p.
27. Hunt R., Cornelissen J.H.C. Components of relative growth rate and their interrelations in 59 temperate plant species. *New Phytol*. 1997; 135: 395–417p.
28. Hoffmann W.A., Franco A.C., Moreira M.Z., *et al.* Specific leaf area explains differences in leaf traits between congeneric savanna and forest trees. *Funct. Ecol*. 2005; 19: 932–40p.
29. Marenco R.A., Siebke K., Farquhar G.D., *et al.* Hydraulically based stomatal oscillations and stomatal patchiness in *Gossypium hirsutum*. *Funct Plant Biol.* 2006; 33: 1103–13p.
30. Carter G.A. Reflectance wavebands and indices for remote estimation of photosynthesis and stomatal conductance in pine canopies. *Remote Sensing of Environ*. 1998; 63: 61–72p.
31. Cornrlissen J.H.C., Lavorel S., Garnier E., *et al.* A handbook of protocols for standardized and easy measurement of functional traits worldwide. *Aus J Bot*. 2003; 51: 335–80p.
32. Wright I.J., Reich P.B., Westoby M. Strategy shifts in leaf physiology structure and nutrient content between species of high and low rainfall and high and low nutrient habitats. *Functional Ecology*. 2001; 15: 423–34p.
33. Kazakou E., Yile D., Shipley B., *et al.* Co-variation in litter decomposition leaf traits and plant growth in species from a mediterranian old field succession. *Functional Ecology*. 2006; 20: 21–30p.
34. Schulze E.D., Kelliher F.M., Korner C., *et al.* Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate, and plant nitrogen nutrition - a global ecology scaling exercise. *Annu Rev Ecol Syst*. 1994; 25: 629p.
35. Shen M., Orcutt D., Foster J.G. Influence of drought on the concentration and distribution of 2 4-diaminobutyric acid and other free amino acids in tissues of flat pea *Lathyrussylvestris L. Environ Exp Bot*. 1990; 30: 497–504p.
36. Pilon-Smits E.A.H., Ebskamp M.J.M., Paul M.J., *et al.* Improved performance of transgenic fructan-accumulating tobacco under drought stress. *Plant Physiol*. 1995; 107: 125–30p.
37. Jaleel C.A., Manivannan P., Lakshmanan G.M.A., *et al.* Alterations in morphological parameters and photosynthetic pigment responses of *Catharanthus roseus* under soil water deficits. *Colloids Surf B: Biointerfaces*. 2008; 61: 298–303p.

38. Sebastiani I., Scebba F., Tongttti R. Heavy metal accumulation and growth responses in poplar clones Eridano (*Populusdeltoides* × *maximowiczii*) and I-214 (*P.* × *euramericana*) exposed to industrial waste. *Environmental and Experimental Botany*. 2004; 52: 79–86p.
39. Hermle S., Lavigne M.B., Bernier P.Y., *et al.* Component respiration, ecosystem respiration and net primary production of a mature black spruce forest in northern Quebec. *Tree Physiol.* 2010; 30: 527–40p.
40. Vögeli-Lange R., Wagner G.J. Subcellular localization of cadmium and cadmium-binding peptides in tobacco leaves. *Plant Physiol.* 1990; 92: 1086–93p.
41. Hartmann T.N., Fricker M.D., Rennenberg H., *et al.* Cell-specific measurements of cytosolic glutathione in poplar leaves. *Plant Cell Environ.* 2003; 26: 965–75p.
42. Reich P.B., Ellsworth D.S., Walters M.B. Leaf structure specific leaf area modulates photosynthesis–nitrogen relations: evidences from within and across species and functional groups. *Functional Ecology*. 1998; 12: 948–58p.
43. Saura-Mas S., Shipley B., Lioret F. Relationship between post fire regeneration and leaf economics spectrum in Mediterranean woody species. *Functional Ecology.* 2009; 23: 103–10p.
44. Zarco-Tejada P.J., Berjo´N. A., Miller J.R. Stress detection in crops with hyperspectral remote sensing and physical simulation models. In: *Proceedings of Airborne Imaging Spectroscopy*, Workshop Bruges, Belgium: Belgian Federal Science Policy, 2004.
45. Jurik T.W. Temporal and spatial patterns of specific leaf weight in successional northern hardwood tree species. *Am J Bot*. 1986; 73:1083–92p.

