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Physiological Aspects of Aluminium Toxicity on Some Metabolic and Hormonal Contents of *Hordeum Vulgare* Seedlings

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Abstract: The aim of this investigation is to throw additional light and integrated view on the role of aluminium, as a toxic element in soil, in altering the growth, hormonal and chemical composition of *Hordeum vulgare* Lcv. Giza 108. The grains were pre soaked in different concentrations of $Al2(SO4)_3$ (0, 10, 2, 0.4 and 0.08µM) for 6 hr, then sown under controlled conditions in plastic pots. Samples were taken for morphological and chemical analysis when plants were 10 and 30 days old. Lower concentrations of Al either steeply (0.08µM) or slightly (0.4 µM) raised all measured growth criteria (mean length of shoot and root, mean number of leaves and lateral roots and mean fresh and dry weight of plants), the contents of each of chlorophyll a, b, total chlorophyll, total pigments, reducing sugars, starch, total sugars, the levels of each of potassium, magnesium, phosphorus, calcium and growth promoters(auxins, gibberellins, cytokinins) and the activity of invertase enzyme whereas higher concentrations (2 and 10µM) obviously reduced them as comparable to untreated plants.On the contrary, Al at lower concentrations decreased the carotenoid and ABA contents and the activity of IAA-oxidase while higher concentrations elevated them.At the other side, there is a positive correlation between the concentration of Al applied and the accumulation of both sodium and iron and the activities of α and β-amylases.

Key words: Aluminium, growth measurements, carbohydrates, photosynthetic pigments, hydrolytic enzymes, phytohormones.

INTRODUCTION

Aluminium is one of the most abundant elements on the earth, constituting about 8% of soil minerals. The acidification of the ground has increased the level of free Al in soils as well as in lakes and there is a positive correlation between the decrease in pH of the lakes and the increasing level of Al in the water (Almer *et al.*, 1978). Natural waters may contain up to 48µM Al (Bingman, 1986). Aluminium (Al^{+3}) is found in approximately 40% of the arable soils of the world (Foy *et al.*, 1978) and acidic soils favor the dissolution of microscopic quantities of Al?³ from metal oxides. Aluminium toxicity is a major factor in limiting growth in plants in most strongly acid soils. Toxic effects on plant growth have been attributed to several physiological and biochemical pathways (Roy *et al.*, 1988). The mechanism of toxicity and resistance to aluminium have been studied and recognized for 70 years. Although the reasons are still unknown (Ma, 2000 and Kachian, 2004), some plant species are more resistant to aluminium than others and variations occur among genotypes of the same species. The uptake of Al into the apoplasm and symplasm is rapid (Lazof *et al.,* 1996; Vazquez *et al.,* 1999) and accordingly various inter and intracellular sites may be affected (Jones *et al*., 1998). Schmohl and Horst (2000) viewed that the damage to plants results mainly from Al accumulation in the root-apoplasm.Extensive work have shown that Al causes an inhibition in root growth (Hodson and Evans, 1994; Graham, 2002 and Jorge and Menossi, 2005), root elongation (Roy *et al*., 1988; Jemo *et al.*, 2006 and Pereira *et al.*, 2006), morphological disorganization in the root apex (Roy *et al.*, 1988), root bending which arose from unequal root cell elongation (Eleftheriou *eta*l,1993) and an alteration in root anatomy (Roy *et al*., 1988; Jemo *et al*., 2006 and Pereira *et al*., 2006). It also inhibits the number and length of lateral roots (Barcelo and Poschenrieder, 2002). Decrease in both shoot growth (Thornton *et al*., 1986 b and c; Graham, 2002) and shoot/root ratio was observed after Al treatment (Hadson and Evans, 1994).Moreover, this element reduces both the fresh weight and dry weight of shoots and roots (Roy *et al*., 1988; Macklon and Sim, 1992; Jemo *et al*., 2006; Sierra *et al*., 2006).Al was reported to induce a reduction in the quantity of chlorophyll pigment and in the

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ratio between chlorophyll a and b which was accompanied by marked decline in photosynthetic rate (Sarkunan *et al*., 1984; Fageria *et al*., 1988). It also suppressed photosystem I mediated electron transport and stimulated photosystem II catalyzed electron flow and O2 evolution (Wavare *et al*., 1983).The total respiratory rate decreased with increased supply of Al in rice, these circumstances were accompanied by a reduction of soluble carbohydrates, including reducing sugars which formed the substrate for respiration (Sarkunan, 1984).Soluble sugars increased in Sorghum when treated with Al up to 2ppm and then remained nearly constant (Cambraia *et al*., 1983a). Graham (2002) indicated that 1mMAl increased the content of sucrose and starch (in stem), but decreased the level of each of glucose, sorbitol, fructose, total soluble carbohydrates, starch and total carbohydrates (in rootsandleaves). Al was found to interfere with certain enzymes governing the deposition of polysaccharides of cell wall (Barber, 1974) and alters the activity of hydrolytic enzymes contained in the Golgi apparatus after being damaged by Al (Roy *et al*., 1988), but no data was available concerning its role on IAA-oxidase enzyme activity. Al was found to reduce Ca uptake in different plants, thus reducing Ca-retention in roots and shoots (Fitter and Hay, 1981 and Sierra *et al*., 2006), it also reduces the sugar maple foliage content of Ca, Mg and K (Berger *et al*., 2001). Cells treated with Al showed lower levels of Na, K, Ca, P, Mg, Fe and Mn (Minocha *eta*l, 1992; Lindberg and Griffiths, 1993). Al has been reported to cause damage to the endoplasm reticulum within the root meristem thus altering its hormone-binding site (Raven and Rubery, 1982). Unilateral application of Al to the root cap, influence the polarity of auxin transport along roots (Hasenstein and Evans, 1988). Moreover, Bennet *et al*. (1990) developed a model for Al-toxicity in which Al could indirectly inhibit (or stimulate) root growth by altering the production and distribution of growth hormones. Recent investigations supported the above view (Barcelo and Poschenrieder, 2002; Massot *et al*., 2002). The present work was carried out: (1) to investigate which concentration of $Al_2(SO_4)_3$ applied is toxic to plants and to which extent it altered both the growth and chemical composition of *Hordeum vulgare* plants. (2) as an approach to understand the physiological mechanism of Al toxicity in plants so as to induce the production of genetically tolerant traits that can overcome its deleterious effect and to be with good performance especially on acid soils.

MATERIALS AND METHODS

Grains of *Hordeum vulgare L.c.v.* Giza 108, were obtained from the Agricultural Research Center, Giza, Egypt. The grains were sterilized with sodium hypochlorite 5% for 5 min, then washed thoroughly with distilled water. They were then soaked for 6 hr at 22°C either in the various concentrations of Al2(So4)3 (10, 2, 0.4, 0.08µM) or in distilled water as control. They were after germinated in plastic pots (15 cm in diameter and 11cm in depth) on Whatman filter paper no. 45 at relative humidity 60-65 %, day length of 12hr, day/night air temperature 22/18°C and light intensity was 3040 Lux. Fifteen seeds were planted in each pot and all pots were arranged into 5 groups (control and the four concentrations selected of Al2(So4)3 each of 8 pots. 15 ml of Hoagland nutrient solution (Hoagland and Arnon, 1950) at pH (5.5±0.2) were added to each pot and renewed every 3 days. After 10 and 30 days of growth, the seedlings were harvested and in the meantime samples were taken so as to be used either immediately for both morphological measurements and pigment extraction, rapidly dried in an oven at 108°C for carbohydrate and mineral determinations or frozen for enzyme and hormonal analysis.

Chemical Analysis:

Photosynthetic pigments (chl.a, chl.b. and carotenoids) were determined spectrophotometrically (Metzener *et al*., 1965).Carbohydrate fractions were extracted and clarified similar to those described by Said and Naguib (1964). The direct reducing sugars (DRS) were determined following the anthrone method suggested by Umbrient *et al*. (1959). The total reducing sugars (TRS) were determined after sucrose hydrolysis and sucrose was calculated from the difference between TRS and DRS.Starch was determined in terms of glucose using the glucose oxidase method after digestion with amyloglucoxidase (Haissig and Dickson, 1979) and the resulted glucose content was then multiplied by 0.9. Enzymes were extracted from plant tissues as adopted by Guerrier and Strullu (1990) with some modifications. IAA-oxidase enzyme was assayed following the method described by Darbyshire (1971), while the invertase activity was assayed following the method adopted by Russel and Jimmy (1980). The activity of α-amylase was assayed according to the procedure adopted by Davis (1977) and it was represented as the decrease in optical density/minute/1gm fresh weight, while the activity of β-amylase was determined following the method described by Malik and Singh (1980). The method of extraction of minerals from plant tissues was essentially similar to that of Chapman and Pratt (1961). Phosphorus was determined following the method described

by Humphries (1956). Sodium and potassium were estimated photometrically according to Williams and Twine (1960). Calcium, magnesium and iron were determined by atomic absorption spectrophotometer according to A.O.A.C. (1984). For estimation of growth hormones, fresh samples were collected and kept in cold redistilled 95% ethanol in which they were after extracted. Then, they were fractionated into aqueous and acidic fractions according to the method described by Shindy and Smith (1975), the acidic fraction contains IAA, GA3 and ABA while the aqueous one contains the cytokinin. Both fractions were finally quantified by HPLC according to the method adopted by Muller and Hilgenbery (1986). Morphological and hormonal data were statistically analyzed according to Snedecor and Cochran (1980). On the other hand, standard deviation (SD) levels have been measured for five replicates of each result of the metabolic analysis.

RESULTS AND DISCUSSION

Growth Parameters: It is evident from Table (1) that the 2 lower concentrations of Al either highly significantly (0.08µM) or significantly and non- significantly (0.4µM) raised all growth criteria which are represented by mean length of root and shoot, mean number of leaves and lateral roots and mean fresh and dry weights of 15 seedlings above those of untreated plants at the two ages of growth. Conversely, increasing Al concentration obviously decreased all this criteria. This inhibitory effect of the higher doses of Al was reported by several authors using various plants. Aluminium was found to induce abnormalities in the root system which include dwarfing of roots (Kerridge *et al*., 1971), reduction or inhibition of the growth of main axis of root with consequent thickening and mottling (Eleftheriou *et al*., 1993; Barcela and Poschenrieder, 2002; Jorge and Menossi, 2005; Jemo *et al*., 2006) former initiation of numerous lateral roots followed by reduction in their growth accompanied by their thickening and browning (Foy, 1984) and finally root bending which arose from unequal cell elongation that results from unequal inhibition of mitotic activity and cell enlargement at both sides of root axis(Eleftheriou *et al*., 1993; Barcelo and Poschenrieder, 2002), Al can interact with multiple sites in the apoplasm and symplasm of root cells. It is located specifically at the root apex. The binding of Al to these sites is probably an important factor in its toxicity (Jaffle *eta*l, 1995; Kochian, 1995; Delhaize *et al*., 2001). Bennet *et al*. (1990) speculated that Al could indirectly inhibit or stimulate root growth (depending on concentration) by altering the production and distribution of growth hormones. Al treatments caused a reduction in shoot growth in several examined plant species (Thornton *et al*., 1986 b and c; Graham, 2002) and in shoot/root ratio (Hodson and Evans, 1994). Al decreases each of dry weight of tops and roots and plant height in rice (Fagria, 1982), shoot fresh anddry weight of cowpea and cucumber (Jemo *et al*., 2006; Pereira *et al*., 2006) and root biomass and leaf area index (LAI)of two tropical maize cultivars (Sierra *et al*., 2006). This trivalent element also resulted in the formation of smaller young leaves that are curled along the margin with yellow tips and having necrotic spots while the growing point collapsed, older leaves show a marginal chlorosis with subsequent lethality (Pavan and Bingham, 1982a; Foy, 1984).

On the contrary, numerous work have indicated that exposure of plants to Al for either a short period (30min to 2hr) or low concentrations, surprisingly, is beneficial for plant growth as it accelerates root formation, root growth and elongation, shoot growth and an overall plant growth stimulation (hormesis) which is consistent with the present results (Matsumoto *et al*., 1979; Barcelo and Poschenrieder, 2002; Pereira *et al*., 2006).Such stimulatory effect of Al at the lower concentrations can possibly be due to either its ability to reduce cell surface negativity which arose from H^+ activity at the membrane surface thereby promoting Fe and P uptake. (Mullette, 1975; Kinraide, 1994; Barcelo and Poschenrieder, 2002 and 2004) or altering distribution of growth regulators in roots (Edwards *et al*., 1976; Barcelo and Poschenrieder, 2002 and Massot *et al*., 2002).

Photosynthetic Pigments: Grain presoaking in the higher concentrations of Al (10 and 2µM) greatly reduced each of chl.a, chl.b, chl (a+b) and total pigments below those of untreated controls. Conversely, treatment with the lower concentrations either produced comparable levels to those of control (0.4µM) or elevated markedly (0.08µM) the pigment amounts at both ages of growth. Concerning, the carotenoid contents, they increased progressively with the increase in the concentration of Al applied (Table 2). The present data was confirmed by different workers who realized remarkable Al- induced reductions in the quantity of chlorophyll pigments (Sarkunan *et al*., 1984) including chlorophyll a and chlorophyll a and b ratio, which was accompanied by degradation of thylakoids in the chloroplast (Pettersson *et al*., 1985) with consequent suppression in photosystem I mediated electron transport whereas photosystem II catalyzed electron flow and O2 evolution was stimulated (Wavare *etal*,1983). Accordingly, photosynthetic rate was declined (Sarkunan *et al*., 1984).Moreover, Pereira *et al.* (2006) demonstrated that Al affects

Table 1: Changes in the growth criteria of *Hordeum vulgare* seedlings in response to aluminium toxicity. (Each value is a mean of ten replicates).

Age/day	Concentration (µM)	Mean length of root		Mean length of shoot		Mean no. of lateral roots		Mean no. of leaves		Mean fresh wt. of 15 seedlings		Mean dry wt. of 15 seedlings	
10	0	5.4		8.3		4.5		1.6		2.58		0.36	
	10	2.1	-HS	4.5	-HS	2.9	-HS	1.0	-HS	2.15	-HS	0.20	-HS
	2	3.6	-HS	5.9	-HS	4.1	NS	1.3	-HS	2.35	-HS	0.25	-HS
	0.4	6.2	+S	7.8	NS	5.7	+HS	1.4	-HS	2.66	+S	0.42	+HS
	0.08	7.3	+HS	9.5	+S	6.2	+HS	1.9	+HS	2.77	+HS	0.51	+HS
L.S.D. at 5%		0.75		0.91		0.86		0.01		0.05		0.06	
L.S.D. at 1%		1.05		1.47		1.08		0.03		0.09		0.10	
30	0	8.3		14.0		7.6		2.8		3.11		0.66	
	10	5.0	-HS	10.9	-HS	6.0	-HS	1.2	-HS	2.85	-HS	0.44	-HS
	2	5.7	-HS	12.2	-S	7.9	NS	1.4	-HS	3.0	-S	0.56	-HS
	0.4	8.1	NS	13.4	+S	8.2	NS	2.2	+HS	3.25	+S	0.73	+S
	0.08	9.8	+HS	16.6	+HS	9.4	+HS	3.3	+HS	3.4	+HS	0.79	+HS
L.S.D. at 5%		0.87		1.03		0.94		0.06		0.07		0.03	
L.S.D. at 1%		1.35		1.91		1.54		0.14		0.16		0.08	

Abbrev. HS: highly significant, S: significant, NS: non-significant

Table 2: Changes in the photosynthetic pigment contents of *Hordeum vulgare* seedlings in response to aluminium treatment. Each value is a mean of 5 replicates and expressed as mg/g. FW. (±SD)

Age/day	Concent-ration(µM)	Chl.a	Chl.b	Chl.(a+b)	Carotenoids	Total Pigments
10	0	3.99±0.3	1.21±0.01	5.2±0.4	1.48±0.01	6.68±0.4
	10	2.78±0.2	1.09±0.02	3.87±0.2	1.81±0.01	5.68±0.2
	2	3.72±0.33	1.33±0.03	5.05±0.3	1.74±0.02	6.79±0.3
	0.4	3.78±0.32	1.69±0.06	5.47±0.2	1.68±0.13	7.15±0.3
	0.08	5.39 ±0.2	1.95±0.02	7.34±0.2	1.27±0.10	8.61±0.4
30	0	8.22±0.36	1.75±0.04	9.97±0.50	1.52±0.14	11.49±0.4
	10	3.01±0.12	2.01±0.003	5.02±0.16	3.31±0.03	8.33±0.01
	2	5.02±0.13	1.51±0.002	6.53±0.18	2.83±0.04	9.36±0.02
	0.4	8.03±0.09	1.82±0.001	8.85±0.20	2.63±0.02	11.48±0.13
	0.08	9.55±0.14	2.03±0.003	11.58±0.17	1.95±0.06	13.43±0.22

chlorophyll synthesis by inhibiting the activity of aminolevulinic acid dehydratase enzyme (ALA-D) responsible for the formation of monopyrrole porphobilonogen which is a part of the chlorophyll molecule as well as the cytochromes and also greatly impairs plant growth.

Carbohydrate Content:

Higher concentrations of Al obviously decreased reducing sugars, starch and total sugar levels below those of untreated plants, while these fractions were raised at the lower concentrations. Sucrose content notably increased in response to the different concentrations applied (Table 3). These results are in agreement with those of Cambraia *et al.* (1983a) who showed that Al up to 2ppm increased soluble sugars in sorghum and then remained constant. They suggested the increase to be due to either reduction in photorespiration (Rodrigues, 1979), hexose phosphorylation (Clarkson, 1966) and cell wall polysaccharide synthesis (HucK, 1972). Al at (1mM) caused severe reductions in reducing sugars, total soluble carbohydrates and total carbohydrates in roots, stem andleaves, increased starch in the root and shoot and increased sucrose amounts in leaves (Graham, 2002). Such increase in the content of soluble sugars which is associated by a decline in starch and total sugars could be attributed to the increased activity of hydrolytic enzymes (α- and β- amylases and invertase) which were estimated, in the present work and a concomitant decline in total pigment amounts and alteration of the chloroplast ultrastructure which eventually resulted in a decline in photosynthetic rate.

Enzyme Activity:

The activities of α and β amylases were directly proportional to the concentrations of Al used (Table 4) and in the mean time above those of control activities at both stages of growth. Lower concentrations of Al (0.4 and 0.08µM) raised the invertase activity while higher concentrations reduced it above and below the control activities respectively. Al, on the other hand, induced a reverse effect on IAA - oxidase activity (i.e., its activity is increased by higher doses and vice versa). In this respect, Barber (1974) found that Al interferes with certain enzymes governing the deposition of cell wall polysaccharides. It also alters the activity of hydrolytic enzymes contained in the Golgi apparatus after being damaged by Al (Roy *et al*., 1988). Unfortunately, no data is available so as to throw

Table 3: Changes in the carbohydrate content of *Hordeum vulgare* seedlings in response to aluminium treatment. Each value is a mean of 5 replicates and expressed as mg/g.DW (±SD)

Age/day	Concent-ration(μM)	Reducing sugars	Sucrose	Starch	Total sugars
10	0	84±3.6	50.4±2.8	441±6.3	709±4.9
	10	62.2±41	53.2±3.1	339±5.6	586±5.2
	2	76.4±2.9	96.4±5.4	441±6.2	693±4.4
	0.4	88.6±3.3	100.8±5.7	543±5.8	784±6.1
	0.08	91.0±5.3	118.0±6.1	573±5.6	956±6.4
30	0	111.2±2.1	69.2±3.2	592±2.3	862±4.4
	10	68.4±1.9	82.4±1.9	294±3.4	488±2.7
	2	83.2±3.4	108.8±2.8	403±4.3	509±3.1
	0.4	109.2±3.3	152.4±2.9	443±3.8	810±5.2
	0.08	128.3±5.1	170.1±3.4	490±4.6	997±5.6

Table 4: Changes in the activities of certain hydrolytic and oxidative enzymes of *Hordeum vulgare* seedlings in response to aluminium treatment. Each value is a mean of 5 replicates and expressed as enzyme activity/g. fresh weight/hour (±SD)

Age/day	Concentration (μM)	α-amylase (decrease in OD/ unit time)	B-amylase (ug maltose released/g.f. wt/h)	Invertase (mg reducing sugar released.g.fwt./h)	IAA-oxidase (ug of IAA oxidised/ g.fwt./h)
10	0	0.46±0.11	32±5.2	425.7±2.2	468.2±1.9
	10	0.29±0.03	70±3.1	354.9±2.4	571.8±1.6
	2	0.34±0.06	56±4.4	388.3±2.8	526.9±1.0
	0.4	0.39±0.09	45±3.4	446.1±2.7	431.8±1.3
	0.08	0.44±0.10	40±3.6	521.4±1.3	382.6±0.2
30	0	0.41±0.12	46±3.2	595.3±2.0	576.3±2.3
	10	0.23±0.03	80±4.7	380.9±0.9	689.2±0.9
	2	0.28±0.05	75±2.9	419.6±1.7	624.7±1.8
	0.4	0.34±0.11	62±2.7	615.2±1.1	517.6±1.1
	0.08	0.38±0.06	55±3.6	666.7±1.4	479.4±1.4

Table 5: Changes in the content of certain mineral elements of *Hordeum vulgare* seedlings in response to aluminium treatment. Each value is a mean of 5 replicates and expressed as mg/g. DW (±SD).

Age/day	Concentration (μM)	Sodium	Potassium	Phosphorus	Magnesium	Calcium	Iron
10	0	301.3±2.3	423.3±1.6	2120.6±3.4	253.6±0.5	412.7±1.3	34.8±0.7
	10	586.9±5.4	308.2±2.3	1496.3±4.1	146.3±1.9	283.8±0.9	79.1±0.8
	2	512.7±3.2	356.6±1.8	1518.9±3.8	187.3±1.6	306.7±1.2	77.2±1.0
	0.4	479.3±2.9	413.6±2.2	1766.8±2.6	218.6±1.1	412.3±0.7	65.8±0.4
	0.08	406.4±2.5	484.7±1.3	2933.7±3.2	289.3±0.8	487.6±0.6	46.9±0.5
30	0	397±3.3	596.3±2.6	3156.2±2.2	326.6±1.4	509.1±1.3	57.7±0.8
	10	733.6±6.2	387.6±2.4	2003.1±3.4	157.4±1.8	366.7±1.2	86.6±0.6
	2	627.1±5.6	438.7±1.9	2638.9±2.6	209.2±1.1	417.6±0.8	79.9±0.7
	0.4	565.8±5.6	554.9±2.1	2719.6±2.9	238.5±0.7	493.1±1.4	73.2±0.5
	0.08	503.4±4.3	602.4±1.7	3426.3±2.4	273.6±1.9	567.4±1.6	68.8±0.8

light on the effect of Al on IAA-oxidase activity. It is proposed that the stimulating effect of Al to hydrolytic enzymes is concurrent with the ability of this element to reduce the membrane permeation to water (Zhao *et al*., 1987; Chen *et al*., 1991; Blamey *et al*., 1993) thus inducing cell water stress. Such condition, in turn, favours the secretion of osmolytic substances as soluble sugars which increases the cell osmotic potential thus forcing more water uptake by the cell (Lutfor Rahman *et al*., 2000 and Xu *et al*., 2002).

Mineral Contents:

Depending on the concentration of Al applied, it either increased the accumulation of each of potassium, phosphorus, magnesium and calcium (0.08μM) or reduced them (10, 2 and 0.4μM) above and below the untreated plants respectively throughout the experimental period (Table 5). Iron and sodium, on the other hand, registered higher amounts at all concentrations of Al used as comparable to those of control amounts. Several reports were obtained that ascertain these results. K uptake was reduced in many tested plants in response to Al treatment (Alam, 1983; Gerzabek and Edelbauer, 1986; Minocha *et al.*, 1992 and Berger *et al.*, 2001), although an increased uptake was observed in others (Lee and Pritchard, 1984 and Thornton *et al.*, 1986a, b and c). Al is proposed to compete with K for root absorption, thus reducing its uptake by roots and thus its content in roots and tops (Alam, 1983 and Berger *et al*., 2001). Increasing Al concentration caused accumulation of P either on the root surface, within the cells or in the free space of roots thus reducing its translocation and therefore its amounts in tops of

various plant species (Greger *et al*., 1992; Barcelo *et al*., 2002; Sierra *et al*., 2006 and Jemo *et al*., 2006). The disturbance in P metabolism by Al resulted in a marked decrease in sugar phosphorylation due to the increased affinity of Al to combine with ATP 40 times that of Mg, thus forming a highly stable Al-ATP complex, thus preventing the transfer of the terminal phosphoryl group to glucose by hexokinase (a Mg-dependent enzyme) (Foy, 1984 and Greger *et al*., 1992). This case alters the respiration rate, the energy production and vitality of treated cells. Moreover, soil-P availability during seedling stage is an important determinant of growth, N2 fixation and grain yield (Vance, 2001).

Extensive results argued with the present work concerning the reduction or accumulation of Mg and Ca due to respectively high and low Al concentrations (Thornton *et al*., 1986 a, b and c and Minocha *et al*., 1992). Al reduces the uptake and transport of these elements thus causing their deficiency symptoms to appear in shoots (Thornton *et al*., 1986 a and b; Hodson and Evans, 1994 and Berger *et al*., 2001). Al competes with Mg at the binding sites of δ-aminolevulinic acid dehydratase enzyme (ALA-D) responsible for the formation of phorphobilongen- a part of chlorophyll molecule as well as the cytochrome molecule, thus affecting the synthesis of pigments which reduces photosynthesis with concomitant reduction in the amount of organic matter and eventually plant growth (Pereira *et al*., 2006). Al is absorbed by cells and competes in an exchangeable manner at almost all calcium binding sites on the cell surface causing the accumulation of hemicellulosic polysaccharides in walls of root tips, this in turn, leads to cell stiffening and thickening that eventually leads to inhibition of root elongation. (Azaizeh *et al*., 1992 and Tabuchi and Matsumoto, 2001). The enhanced accumulation of Fe and Na in *Hordeum* in response to Al treatment was supported by (Alam, 1983; Minocha *et al*., 1992 and Berger *et al*., 2001), although other studies showed that Al lower the absorption of Fe (Cambraia *et al*., 1983a and Gerzabek and Edelbauer, 1986).

Such increase in Na values can be considered as one of the tools that Al-treated plants would lead in order to increase the negative osmotic potential of tissues that arose from the reduction in membrane water permeability thus increasing the ability of cells and tissue for water and solute uptake from soil (Rodriguez *et al*,1996).Regarding the increasing level of Fe, it can be attributed to the corresponding increases in peroxidase activities in *Hordeum* plant (Abdalla,under press) or it may be due to the stimulation of Al to the radical chain reactions mediated by iron ions so as to enhance lipid peroxidation (Yamamoto *et al*., 2001).

Phytohormones:

The changes in the phytohormonal levels of untreated and Al-treated plants are presented in Figs. (1, 2, 3 and 4). Depending on the dose of Al applied, the contents of each of auxins, gibberellins and cytokinins were either increased (0.08µM) or decreased (at higher concs.) above and below the control levels respectively at the two ages of plant growth, while the ABA contents were reversibly increased progressively with the increase in the concentration of Al used. Similar results were reported by Raven and Rubery (1982), Bennet *et al*. (1990) and Barcelo *et al*. (2002) and they speculated that Al at certain concentration could indirectly inhibit or stimulate root growth by altering the production and distribution of growth hormones. Hasenstein and Evans (1988) demonstrated that unilateral application of Al to the root cap could influence the polarity of auxin transport along roots. It may also inhibit the basipetal auxin transport from root meristem to elongation zone resulting in decreased root cell elongation (Kollmeier *et al*., 2000). Recent investigations suggest that ethylene may be involved in fast signal transduction of Al-induced enhancement of cytokinin levels in roots. These suggestions were supported by the finding that Al-induced transient rise in ethylene production in roots after 5min of Al exposure which was followed after 15min by a substantial increase of root cytokinin levels of beans (Massot *et al*., 2002). Another view postulated that Al causes root inhibition through alterion of hormone gradients within the root meristems as a consequence of damage to the endoplasmic reticulum, which is a hormone- binding site (Raven and Rubery, 1982). Thus with progressive increasing environmental metal load and consequent acid rain, soil acidification is enhanced and Al plays a major role in the loss of specific tree species as well as loss of total vegetational cover at specific sites. Consequently, extensive work has been done during the last decade to elucidate the threshold of Al toxicity as a function of either its exposure time or doses applied, its mechanism of action on plants and the mechanism of plant tolerance to it. Accordingly, in the present work, Al treatment either shows hormetic or toxic effects at respectively low and high doses. It is hypothesized that toxic concentrations of Al formerly, induced alterations in hormonal levels in roots (by either decreasing the biosynthesis of growth promoters or inhibiting their translocation from the root meristem to the elongation zone, beside increasing the level of growth inhibitors), thus causing cell wall

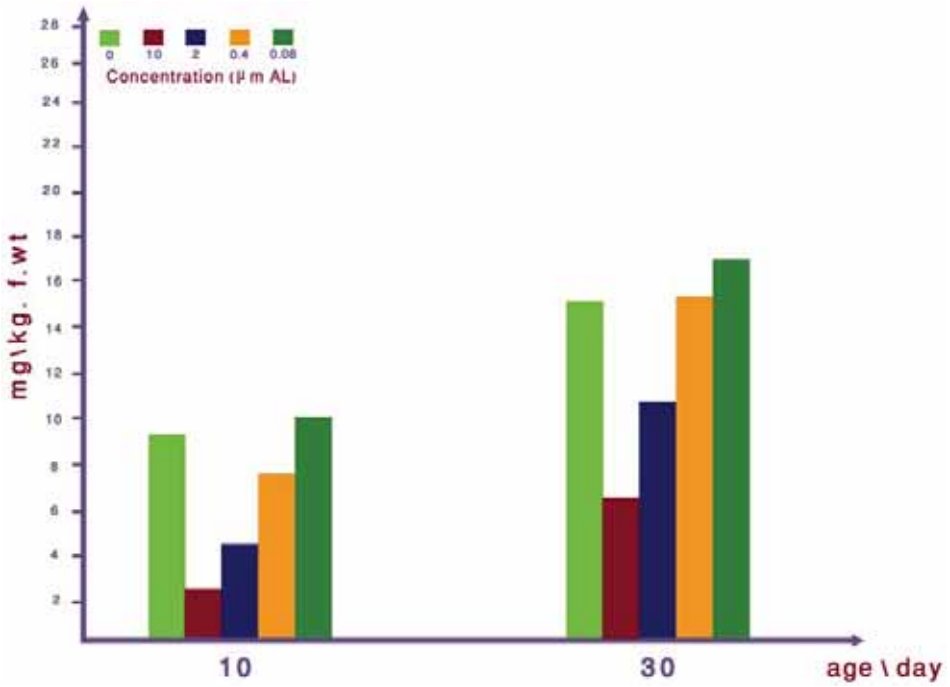

Fig. 1: Changes in the auxin content of *Hordeum vulgare* seedling in response to AL treatment

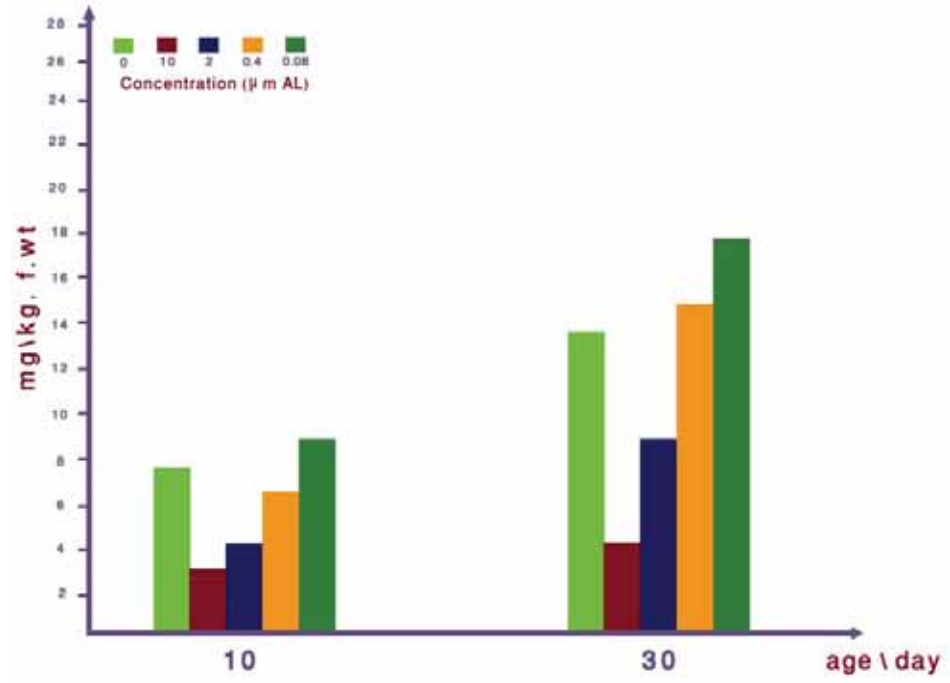

Fig. 2: Changes in the gibberellin content of *Hordeum vulgare* seedling in response to AL treatment

stiffening and thickening leading to inhibition of cell elongation and growth (Kollmeier *et al*., 2000; Gunse *et al*., 2000 and Massot *et al*., 2002). In addition, to the inhibition of root growth, Al treatment also affects plant growth by impairing metabolic activity reducing chlorophyll synthesis, photosynthesis, respiration and

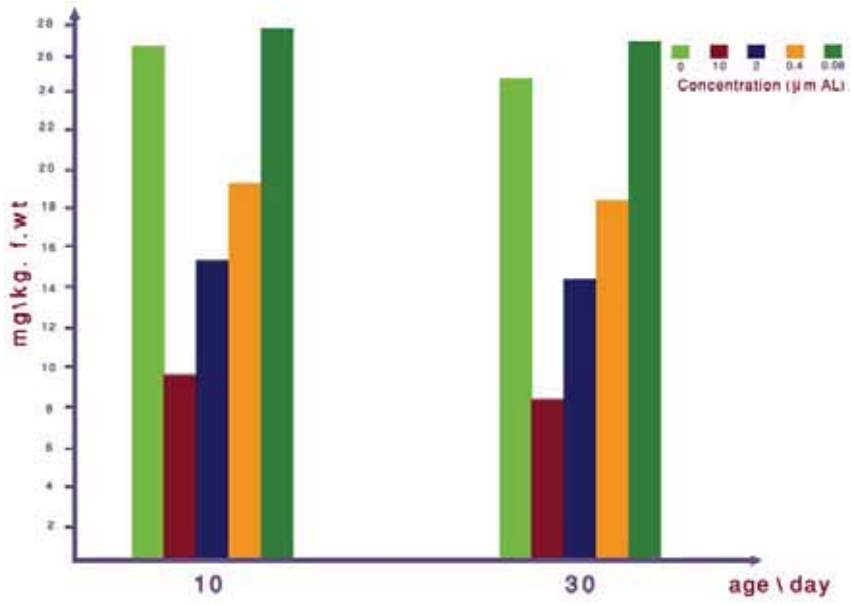

Fig. 3: Changes in the cytokinin content of *Hordeum vulgare* seedling in response to AL treatment

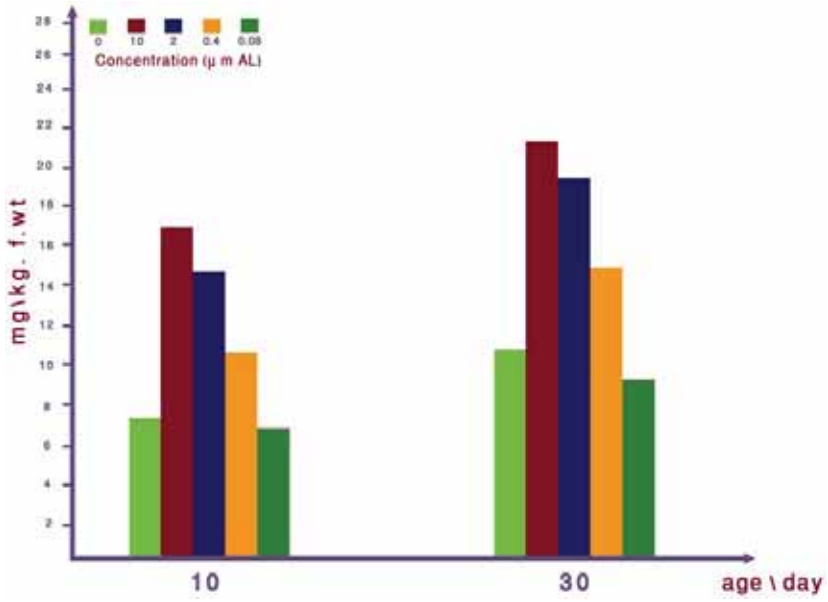

Fig. 4: Changes in the MBA content of *Hordeum vulgare* seedling in response to AL treatment

carbohydrate contents (De Lima and Copeland, 1994), altering nutrient availability in the rhizosphere, nutrient uptake and translocation by plants (Matsumoto, 2000) and water uptake (Blamey *et al.*, 1993). It eventually causes extensive plasma membrane damage, peroxidation of membrane lipids and loss of cell compartmentation (Ishikawa and Wagatsuma, 1998 and Barcelo *et al*., 2002).

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