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Interactive effect of heavy metals and temperature on the growth, and chlorophyll, saccharides and soluble nitrogen contents in *Phaseolus* plants

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Abstract

The effect of combinations of different concentrations of copper (Cu) and mercury (Hg) ions with different constant temperatures (T) on *Phaseolus vulgaris* plants was studied. Criteria investigated included shoot and root length, dry mass, chlorophyll content (Chl) and stability index (CSI), and contents of soluble (SS) and hydrolysable saccharides (HS), soluble proteins (SP) and total free amino acid (AA). Each of the factors (T, Cu and Hg) significantly affected the parameters tested with exception of T effect on Chl *b* content as well as on Chl *a*/*b* ratio. Bifactorial interactions (T × Cu) or (T × Hg) were also significant, except the interaction (T × Hg) in shoot elongation, Chl *b* content and Chl *a*/*b* ratio. Statistical treatment of the data lead to three findings: (*1*) temperature was dominant in affecting CSI*a*, shoot AA and root SS, (*2*) Cu and Hg had the predominant effect on growth parameters and Chl content, and (*3*) interactions (T × Cu and T × Hg) were dominant in affecting CSI*b*, shoot SP, and root HS.

Introduction

Multifactorial experiments and elucidation of interactions of individual effects are an important goal in biological studies. Temperature (T) is one of the most important environmental factors that limit plant distribution (*e.g.* Woodward 1987) and productivity (*e.g.* Hay and Walker 1989). The acclimation of plants to grow at low T involves a variety of morphological and biochemical changes (Levitt 1980, Long and Woodward 1988). Interaction of this factor with effects caused by heavy metals is very important. Heavy metal ions accumulate in different parts of the plant after they are readily absorbed by the root system, resulting in retardation of plant growth (Påhlsson 1989, Van Steveninck *et al.* 1990). This could be due to their interference with activities of enzymes essential for normal metabolic and development processes (Nath 1986, Van Asshe and Clijsters 1990) and for various photosynthetic functions

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(Clijsters and Van Assche 1985, Lidon and Henriques 1992, 1993, Mohanty and Mohanty 1988, Stoyanova and Tchakalova 1993). An important harmful effect of mercury and copper is the alteration of plasma membrane permeability of cells, leading to leakage of potassium and other solutes (Passow *et al.* 1961, Wainwright and Woolhouse 1977, De Filippis 1979, Ohsumi *et al.* 1988).

The aim of the present work was to study the interactive effects of Cu and Hg and T on growth, Chl content and Chl stability to heat of *Phaseolus* plants. In addition, contents of soluble (SS) and hydrolysable saccharides (HS), soluble proteins (SP) and total free amino acids (AA) were studies.

Materials and methods

Bush bean seeds (*Phaseolus vulgaris* L. cv. Contender) were germinated in trays of moist perlite, in controlled environmental chambers irradiated by "cool white" fluorescent lamps. The irradiation was 400 - 450 μmol m^{-2} s^{-1} near the primary leaves of the germinated plants. The photoperiod was 12 h. The day/night T and relative humidity were 25/23 °C and 70/75 %, respectively.

Seven days after sowing, the seedlings were selected for uniformity and transferred to plastic pots (five per pot) filled with continuously aerated nutrient solution containing [mol m^{-3}]: KNO_3 1.5, $Ca(NO_3)_2.4H_2O$ 1.0, $NH_4H_2PO_4$ 0.5, $MgSO_4.7H_2O$ 0.25, KCl 0.05, H_3BO_4 0.025, FeNa EDTA 0.02, $MnSO_4.H_2O$ 0.002, $ZnSO_4.7H_2O$ 0.002, $CuSO_4.5H_2O$ 0.0005, and $(NH4)_6Mo_7O_{24}$ 0.0005, pH 5.5. When the seedlings were 2 weeks old (22 d from sowing) the nutrient solution was replaced by solutions containing different concentrations (0, 1, 10, 100 and 1000 μM) of Cu or Hg ions. Sulphate salt of copper and chloride salt of mercury were used. The plants were classified into three groups and exposed to different constant temperatures (15, 25 and 35 °C). Root temperature was allowed to follow shoot ambient temperatures. Three pots were assigned at random to each treatment combination at every metal concentration. The plants were kept under treatment for further one week prior to analysis.

Chl *a* and *b* contents were determined according to Todd and Basler (1965). Chl stability index (CSI) was assessed according to Murty and Majumder (1962): the samples were heated at 60 °C for 1/2 h in water bath and CSI was determined as Chl content in heated sample/Chl content in fresh sample × 100.

For growth response determination, the plants were removed carefully from the pots and the roots were washed thoroughly. Shoot and root lengths were measured and the plant material was dried at 70 °C to constant mass.

SS, HS, AA and SP in plant extracts were determined according to Dubois *et al.* (1956), Pucher *et al.* (1948), Lee and Takahanshi (1966) and Lowry *et al.* (195l), respectively.

Statistical evaluation included analysis of variance and coefficient of determination (η^2). The latter evaluates the relative effect of each single factor and its interaction in contributing to the total response (Ostle 1963, El-Sharkawi and Springuel 1977).

Results

Growth response: The growth of *Phaseolus* plants as indicated by shoot length, root length and dry mass of shoot and root was reduced by Cu and Hg ions at the three temperatures tested (Table 1) except at low T (15 °C) when both metal ions stimulated root elongation at low concentrations (1 and 10 μM). Root elongation and dry mass production in both shoot and root were best at 15 °C. The effects of T, Cu, Hg, and their interactions on shoot and root elongation and dry mass production was mostly highly significant. The T × Hg interaction had no significant effect on shoot length. The magnitude of the relative effects of each factor and factorial interaction on growth parameters (as indicated by η^2 values) showed that the relative role of Cu or Hg was predominant but that of temperature was subdominant and the role of interaction was only a minor one.

Chl content, Chl *a/b* ratio and CSI: Chl content was affected by the heavy metal ions at the three constant temperatures tested (Table 2). While Chl *a* and *b* contents markedly declined with increasing external concentrations of Cu and Hg at 25 and 35 °C, the Chl content was slightly enhanced by 100 μM Cu solution at 15 °C. Both Chl *a* and *b* contents were much higher in metal-treated plants at 15 °C than at either 25 or 35 °C. The Chl *a/b* ratio was mostly reduced by either Cu or Hg at temperatures from 15 to 35 °C. Only at 15 and 25 °C the ratio was higher than in the control plants at Cu concentration lower than 10 μM. Addition of Cu and Hg at low and high temperatures (15 and 35 °C, respectively) decreased Chl *a* and *b* stability to heat (Table 2). Only 10 μM concentration of Cu ions increased CSI*b* at 15 °C. The same was true for Chl *a* and *b* stability at 100 μM Hg ions and 35 °C. At 25 °C CSI*b* was increased by Cu and Hg treatment, while CSI*a* was decreased (10 and 100 μM concentration of Cu ions was an exception for CSI*a*). Temperature of 25 °C was best for increases of CSI in Cu-treated plants, while in Hg-treated plants it was 35 °C. The effects of T, Cu and Hg as well as their interaction on Chl content, Chl *a/b* ratio and CSI were mostly highly significant (statistically insignificant was only the effect of T and T × Hg interaction on Chl *b* content and Chl *a/b* ratio). The relative role of heavy metal ions in affecting Chl content and Chl *a/b* ratio was dominant and that of T and interaction was subdominant. Temperature had predominant effect on CSI a while the role of interaction was subsidiary and that of heavy metals was a minor one. The share of interaction was predominant in CSI*b*.

Saccharides: The content of shoot SS was higher in metal-treated plants than in control at all temperatures (Table 3). Only 1000 μM concentration of Hg ions partially decreased shoot SS at 15 and 35 °C. Root SS content tended to parallel that of shoot SS in response to Cu treatment at 15 and 25 °C. At 35 °C, the content of root SS increased at l μM Cu and decreased again at higher concentration. Addition of Hg decreased the SS content in roots at 15 °C. The reverse was found at 25 °C. At 35 °C the content of root SS increased at 1 to 10 μM Hg but it decreased at higher doses. In general, maximum accumulation of SS in both shoot and root was observed at 15 °C in the presence or absence of metal ions.

Table 1. Growth response of *Phaseolus vulgaris* to varying external concentrations of Cu and Hg ions at different temperatures.

Temperature [°C]	Concentration [μM]	Length [cm] Cu shoot	root	Hg shoot	root	Dry mass [g] Cu shoot	root	Hg shoot	root
15	0	20.87±0.34	18.00±0.38	20.87±0.34	18.00±0.38	1.67±0.03	0.35±0.030	1.67±0.03	0.35±0.030
	1	16.67±0.57	20.73±0.90	14.60±0.38	18.07±0.14	0.88±0.02	0.21±0.010	0.74±0.04	0.21±0.020
	10	14.53±0.69	19.53±1.14	14.80±0.25	19.20±1.00	0.66±0.05	0.20±0.005	0.62±0.04	0.16±0.005
	100	9.75±0.72	14.53±0.33	13.51±0.44	15.87±1.01	0.44±0.04	0.16±0.010	0.64±0.01	0.20±0.020
	1000	8.80±0.33	14.13±0.85	10.20±0.68	12.40±0.25	0.41±0.03	0.09±0.010	0.47±0.06	0.15±0.020
25	0	25.40±0.68	19.95±0.27	25.40±0.68	19.95±0.27	1.51±0.01	0.36±0.040	1.51±0.01	0.36±0.040
	1	24.93±0.61	13.47±0.64	15.33±0.64	16.73±0.55	0.73±0.06	0.11±0.009	0.72±0.02	0.18±0.007
	10	21.53±0.22	13.67±0.98	15.35±0.80	16.60±0.25	0.60±0.01	0.11±0.009	0.47±0.04	0.16±0.005
	100	19.13±0.55	12.00±0.34	13.80±0.57	15.40±0.38	0.46±0.03	0.14±0.007	0.59±0.02	0.20±0.020
	1000	8.47±1.31	6.13±0.67	10.66±0.43	14.07±0.48	0.20±0.03	0.08±0.008	0.39±0.02	0.12±0.003
35	0	27.93±1.94	15.55±1.06	27.93±1.94	15.55±1.06	1.18±0.04	0.13±0.010	1.18±0.04	0.13±0.010
	1	21.67±0.79	16.55±0.33	17.73±0.85	8.53±0.90	0.70±0.04	0.10±0.005	0.56±0.05	0.08±0.005
	10	19.85±0.99	10.60±0.57	17.67±0.29	10.27±0.54	0.44±0.02	0.08±0.007	0.56±0.04	0.11±0.003
	100	19.93±0.63	8.67±0.11	13.73±1.01	5.80±0.33	0.57±0.09	0.08±0.005	0.33±0.05	0.07±0.007
	1000	9.13±0.52	7.20±0.41	12.45±1.16	3.93±0.20	0.31±0.02	0.09±0.007	0.38±0.05	0.05±0.002

Table 2. Chlorophyll (Chl) content [g kg^{-1}(f.m.)], Chl *a*/*b* ratio and Chl stability to heat (CSI) [%] in *Phaseolus vulgaris* at different Cu and Hg ion concentrations and different temperatures.

	Conc. [μM]	15 °C Cu	15 °C Hg	25 °C Cu	25 °C Hg	35 °C Cu	35 °C Hg
Chl *a*	0	1.12±0.02	1.12±0.02	1.11±0.04	1.11±0.04	1.23±0.10	1.23±0.10
	1	1.00±0.04	0.42±0.06	0.63±0.07	0.46±0.01	0.54±0.07	0.57±0.15
	10	1.12±0.02	0.67±0.06	0.30±0.03	0.40±0.01	0.65±0.05	0.64±0.13
	100	1.24±0.06	0.51±0.09	0.31±0.05	0.47±0.03	0.46±0.10	0.32±0.05
	1000	0.67±0.07	0.37±0.05	0.28±0.02	0.19±0.01	0.28±0.04	0.18±0.04
Chl *b*	0	0.63±0.03	0.63±0.03	0.77±0.11	0.77±0.11	0.74±0.06	0.74±0.06
	1	0.53±0.02	0.30±0.03	0.35±0.05	0.31±0.01	0.33±0.04	0.38±0.07
	10	0.62±0.02	0.53±0.06	0.22±0.02	0.31±0.02	0.47±0.02	0.52±0.12
	100	0.74±0.04	0.36±0.06	0.22±0.04	0.38±0.05	0.33±0.08	0.24±0.02
	1000	0.44±0.04	0.30±0.04	0.20±0.01	0.24±0.05	0.36±0.08	0.19±0.02
Chl *a*/*b*	0	1.77±0.05	1.77±0.05	1.50±0.20	1.50±0.20	1.66±0.01	1.66±0.01
	1	1.90±0.03	1.36±0.09	1.81±0.02	1.48±0.03	1.63±0.02	1.46±0.23
	10	1.82±0.01	1.27±0.03	1.37±0.12	1.29±0.05	1.38±0.05	1.24±0.05
	100	1.67±0.02	1.42±0.07	1.43±0.08	1.27±0.18	1.43±0.10	1.33±0.07
	1000	1.51±0.07	1.24±0.09	1.21±0.04	0.81±0.11	0.72±0.11	0.95±0.18
CSI*a*	0	65.86±1.99	65.86±1.99	80.42±1.20	80.42±1.20	79.52±1.81	79.52±1.81
	1	47.35±2.86	55.67±0.87	67.01±1.96	61.59±0.92	48.68±1.11	72.86±0.68
	10	66.48±1.09	37.38±0.54	84.67±0.45	74.19±1.18	42.45±0.78	79.27±1.15
	100	45.36±1.87	62.43±1.20	64.14±1.05	57.68±2.92	59.49±0.65	84.39±1.41
	1000	44.72±2.22	53.29±1.34	93.91±0.91	66.51±1.46	75.53±1.43	86.28±2.23
CSI*b*	0	76.72±1.11	76.72±1.11	66.65±0.90	66.65±0.90	89.79±0.81	89.79±0.81
	1	67.91±2.13	65.81±0.91	98.96±0.56	92.35±2.39	68.93±1.79	83.35±0.61
	10	94.33±0.66	40.45±0.90	95.07±1.00	96.00±1.31	54.38±1.85	79.05±1.28
	100	52.22±0.56	63.28±2.19	79.53±0.67	83.15±1.71	76.75±0.78	93.37±1.20
	1000	61.91±0.78	75.64±0.89	69.68±0.78	68.28±0.99	71.55±4.19	76.85±0.87

Cu and Hg treatments reduced shoot HS content at the tested temperatures except at 25 °C when Cu-treated plants contained more shoot HS than the control. At low T, the root of *Phaseolus* accumulated less HS than in control at relatively low Cu concentration (1 and 10 μM) but the accumulation was increased at higher concentration. The reverse was true at high T. Hg had a similar effect on root HS content at high and low T, raised it at low concentration, but decreased it at higher doses. 25 °C was optimal for HS accumulation in shoots of metal-treated plants.

T, Cu and Hg and their interactions had highly significant effects on saccharide contents. The relative role of the T × Cu interaction was predominant for shoot and root HS, while the role of T and Cu was subdominant. The T × Hg interaction was predominant for root SS. T and the T × Hg interaction were nearly equally dominant for shoot SS contents ($\eta^2 = 0.38$ and 0.36, respectively).

Table 3. Changes in contents of soluble saccharides (SS) and hydrolysable saccharides (HS) in *Phaseolus vulgaris* caused by different Cu and Hg ion concentrations at different temperatures.

Temperature [°C]	Concentration [μM]	SS [g kg^{-1}(d.m.)] Cu shoot	Cu root	Hg shoot	Hg root	HS [g kg^{-1}(d.m.)] Cu shoot	Cu root	Hg shoot	Hg root
15	0	43.66±1.31	37.88±1.16	43.66±1.31	37.88±1.16	32.06±1.32	4.60±0.15	32.06±1.32	4.60±0.15
	1	67.51±0.87	47.09±0.60	49.79±0.66	26.91±0.81	22.27±0.92	3.40±0.22	3.03±0.21	15.02±0.58
	10	60.09±1.02	56.77±1.20	55.58±0.87	31.52±1.15	16.38±0.72	2.56±0.22	10.75±0.29	9.13±0.64
	100	63.99±1.09	43.17±1.19	49.96±0.74	22.89±1.34	13.51±0.55	8.64±0.58	11.43±0.51	2.38±0.13
	1000	49.17±0.87	24.60±1.17	24.94±0.80	28.41±1.10	12.00±0.92	7.49±0.48	0.79±0.04	1.06±0.02
25	0	15.09±0.55	13.07±0.15	15.09±0.55	13.07±0.15	12.65±0.30	13.47±0.63	12.65±0.30	13.47±0.63
	1	41.94±0.58	29.30±0.63	30.66±0.85	27.35±0.55	29.57±0.51	6.43±0.58	15.12±0.50	1.95±0.29
	10	47.44±1.17	15.19±0.60	36.05±1.14	11.51±0.74	34.47±0.58	5.25±0.55	1.61±0.14	0.63±0.03
	100	33.24±1.44	23.77±0.94	40.31±1.44	45.76±0.84	39.87±0.56	2.97±0.55	2.64±0.28	14.42±0.70
	1000	23.81±1.73	9.54±0.58	35.31±1.11	43.53±1.03	17.11±1.22	6.82±0.23	2.60±0.22	4.72±0.36
35	0	25.26±0.54	16.26±0.31	25.26±0.54	16.26±0.31	20.94±1.11	1.44±0.20	20.94±1.11	1.44±0.20
	1	69.31±0.60	28.06±0.72	43.04±1.49	31.05±1.17	2.47±0.25	15.14±0.55	2.93±0.04	8.53±0.46
	10	33.02±1.74	10.33±0.57	28.64±0.92	20.46±0.44	4.14±0.11	3.44±0.26	12.10±0.58	6.64±0.60
	100	36.43±1.50	14.34±0.74	21.36±0.77	8.52±0.70	5.71±0.20	0.41±0.06	3.74±0.22	0.80±0.09
	1000	21.47±0.78	11.95±1.09	19.79±0.85	7.93±0.99	8.09±0.19	0.95±0.04	0.72±0.06	1.79±0.08

Table 4. Changes in contents of soluble proteins (SP) and total free amino acids (AA) in *Phaseolus vulgaris* caused by different Cu and Hg ions concentrations at different temperatures.

Temperature [°C]	Concentration [μM]	SP [g kg^{-1}(d.m.)] Cu shoot	root	Hg shoot	root	AA [g kg^{-1}(d.m.)] Cu shoot	root	Hg shoot	root
15	0	20.42±0.77	23.67±0.51	20.42±0.77	23.67±0.51	12.69±0.33	5.41±0.25	12.69±0.33	5.51±0.25
	1	36.00±1.15	45.90±0.81	46.80±0.82	25.50±0.87	6.63±0.69	4,32±0.64	3.87±0.37	2.93±0.36
	10	47.16±1.25	26.81±0.68	64.24±0.52	22.81±0.61	7.76±0.83	4.45±0.66	7.33±0.29	2.76±0.38
	100	45.04±0.74	43.55±0.78	55.14±0.87	23.03±0.74	9.71±0.33	2.33±0.20	10.00±0.16	7.03±0.30
	1000	34.13±1.15	10.63±0.29	34.67±0.89	10.49±0.35	10.38±0.60	1.48±0.23	11.43±0.27	1.18±0.10
25	0	15.57±1.08	8.88±0.13	15.57±1.08	8.88±0.13	4.24±0.32	7.68±0.58	4.24±0.32	7.68±0.58
	1	27.50±0.87	19.89±0.58	38.24±0.57	22.56±0.53	3.84±0.36	2.47±0.26	4.50±0.29	0.66±0.01
	10	23.00±1.15	14.05±0.89	48.50±0.87	33.84±0.84	8.51±0.36	1.13±0.03	5.50±0.29	3.17±0.10
	100	51.13±0.58	16.50±0.54	56.65±1.86	34.84±0.92	12.77±0.61	1.04±0.02	6.00±0.58	1.43±0.23
	1000	42.33±1.05	10.25±0.50	44.16±1.17	27.24±0.85	8.14±0.85	1.57±0.06	11.21±0.58	1.61±0.06
35	0	37.40±0.37	15.75±0.58	37.40±0.37	15.75±0.58	14.48±0.61	6.84±0.30	14.48±0.61	6.84±0.30
	1	32.67±0.84	27.63±0.87	39.62±0.79	26.98±0.64	19.27±0.61	8.33±0.57	2.31±0.59	0.74±0.08
	10	34.82±0.97	17.01±0.65	45.24±0.86	23.79±0.89	22.63±0.92	3.65±0.30	13.06±0.09	1.46±0.14
	100	41.06±1.49	8.58±0.32	49.67±0.89	20.81±0.76	12.55±0.82	2.22±0.12	18.44±0.59	2.33±0.17
	1000	24.85±0.88	4.77±0.42	34.91±0.81	11.43±0.69	8,76±0.78	1.32±0.06	7.18±0.29	0.18±0.01

Soluble nitrogen: SP content in shoots (Table 4) was enhanced by Cu and Hg treatments at the three tested temperatures. Only higher concentration (1000 μM) of metal ions decreased the protein content at high T compared to control. The content of root SP was enhanced by Cu treatment at 15 and 25 °C and suppressed at Cu concentration greater than 10 μM at 35 °C. Mercury increased SP content in root at 25 and 35 °C. The same was true at concentrations larger than 10 μM at 15 °C. Accumulation of SP was high at 15 and 35 °C particularly at low metal ion concentrations, compared to that at 25 °C.

Shoot total free AA content (Table 4) was lower in metal-treated plants than in untreated control at 15 °C. The reverse was true at 25 °C. At 35 °C the shoot AA content increased at 1 to 10 μM Cu concentration, but decreased again at the higher concentration. Root AA content was lower in metal-treated plants than in the control at the three tested temperatures except at 100 μM Hg at 15 °C and 1 μM Cu at 35 °C.

The effect of the tested factors and their interactions on SP and total free AA contents were highly significant. The relative role of T × Cu interaction in SP accumulation in shoots was dominant. T and Cu were equally dominant for contents of root SP ($\eta^2 = 40$). Cu and Hg were dominant for root AA acids while the role of T was subsidiary.

Discussion

The reduction in growth induced by Cu and Hg (Table 1) could be a consequence of their interference with a metabolic processes associated with normal development especially (*1*) synthesis of proteins (Stiborová *et al.* 1987), (*2*) activities of some important enzymes by binding to free amino carboxyl or side groups and/or replacing of some important metal ions associated with such groups (Nath 1986, Stiborová *et al.* 1987, Påhlsson 1989, Van Asshe and Clijsters 1990) and (*3*) various photosynthetic processes like Chl biosynthesis (Stobart *et al* 1985), activities of photosystems and photophosphorylation or electron transport (Murthy *et al.* 1990, Lidon and Henriques 1992). A stimulatory effect of low concentrations of Cu or Hg (0 to 10 μM) in promoting root elongation of plants at low T (15 °C) could be due to their ability to act as an activator of important enzymes. Such response indicates that the inhibitory effect of these metal ions on growth may be nullified or reversed under low T.

Chl content decrease induced by heavy metal ions at the three tested T (Table 2), could be due to inhibition of Chl synthesis (Stiborová *et al.* 1987) and/or to inhibition of synthesis of 5-aminolaevulinic acid and of the protochlorophyllide reductase activity (Stobart *et al.* 1985). At high and low T both metals decreased Chl stability to heat probably through increasing Chl degradation by heat.

Soluble sugars content was higher in shoot and root of metal-treated plants than in untreated ones especially at 15 and 35 °C. This increase may be an adaptive response of plant which involves maintainance of favourable water balance. Since both Hg and Cu inhibit Chl synthesis and photosynthetic activity (Stobart *et al.* 1985) and reduce CO_2 fixation (Stiborová *et al.* 1987), the increase in SS content could be due to

metal-induced acceleration of HS interconversion to SS through activation of enzymes. The content of shoot HS (Table 3) was reduced by both metal ions at 15 and 35 °C. At 25 °C the content of shoot SS and HS were higher in Cu-treated plants than in the control. Thus Cu ions did not inhibit photosynthetic activity and did not reduce shoot SS and HS at these temperatures.

The SP content in shoot and roots was effectively increased by the tested ions (Hg was an exception in root SP at 15 C). The increase in SP may be due to accumulation of proline under heavy metal stress (Alia and Pardha Saradhi 1991) which is important in osmoregulation (Ahmed and Hellebust 1988, Laliberte and Hellebust 1989), protects enzymes against denaturation (Paleg *et al.* 1984, Nikolopoulos and Manetas 1991), acts as reservoir of carbon and nitrogen sources (Fukutaku and Yamada 1984), and stabilizes the machinery of protein synthesis (Kadpal and Rao 1985). The enhancement of SP accumulation by metal action is an adaptive response to improve water retention properties. Total AA content remained low in shoots and roots of metal-treated plants, probably due to enhanced incorporation of AA in protein synthesis.

Temperature, Cu and Hg as well as their interactions had a highly significant effects on most parameters tested. The coefficient of determination (η^2) indicated that T, Hg and Cu and their interactions had dual roles in their dominant and subsidiary effects. The interaction between heavy metal ions and T was confirmed by our results. The relative role of single factors was minor, even if significant. Thus the responses of tested parameters to heavy metal treatment were different under various T, sometimes the inhibitory effect of metal ions was even nullified or reversed by temperature.

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