RESEARCH ARTICLE

Physiological responses of *Eichhornia crassipes* [Mart.] Solms to the combined exposure to excess nutrients and Hg.

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ABSTRACT

In the present study the physiological response of water hyacinth (*Eichhornia crassipes* [Mart.] Solms) to the combined exposure of excess nutrients and Hg was examined. Young water hyacinth plants were exposed to a range of $HgCl_2$ and KNO_3 concentrations. After eight weeks, submerged plant tissues reached an Hg concentration of 4 mg g^{-1}. The accumulation of P and S was reduced by the addition of $HgCl_2$, and also the P and K concentrations in emerged and submerged parts respectively decreased. In contrast, the addition of $HgCl_2$ increased Ca and Mg concentrations in submerged parts. Furthermore, the concentration ratios of submerged/emerged parts for Ca, Mg and P were also reduced by the addition of $HgCl_2$. The interaction of $HgCl_2$ and KNO_3 was synergistic and decreased F_v/F_m, total chlorophyll content and P and Mn concentrations in emerged parts. In submerged plant parts Ca concentration increased and K content stabilized as a result of the above interaction. However, the total accumulation of Hg per plant was not reduced, thereby confirming the water hyacinth as a promising candidate for the heavy metal phytoremediation of eutrophic waters.

Key-words: Abiotic stress, metal accumulation, multiple stress, phytoremediation, water eutrophication, water pollution.

INTRODUCTION

Mercury is a non-essential heavy metal that is toxic to living organisms in very small doses, strongly persistent in the environment and readily transferred to the food chain (Leady and Gottgens, 2001). Its properties have motivated the United States Environmental Protection Agency to recognise it as a serious risk for human health and the environment (USEPA, 1997). Mercury contamination of surface waters results from atmospheric deposition, erosion, urban discharges, mining activities, combustion and industrial sources (e.g. chlor-alkali industries), and agricultural run-off (Wang et al., 2004). Affected water bodies are usually near urban areas, where increasing amounts of nutrients, mainly N, are released by human activities, and eutrophication is common. Nutrient-enriched environments can reduce heavy metal uptake by plants as a consequence of competition with nutrient cations, or binding to nutrient anions (Greger, 1999).

In addition, the effects of combined exposure to excess nutrients and heavy metals may not be simply additive, as the interaction of two stresses is unique and cannot be inferred from studying their individual effects (Voesenek and Pierik, 2008). To the best of our knowledge, few studies report the response of macrophytes to the combined effect of heavy metals and nutrient excess. Despite this, a number of papers from different areas such as ecology and water treatment address this subject (Lim et al., 2003; Nimpstch et al., 2005).

The water hyacinth (*Eichhornia crassipes* [Mart.] Solms) is a floating macrophyte native to the Amazon basin. Brazil is the most likely place of its origin, with a natural extension to other areas of the South American continent. The beauty of its flowers led to the introduction of *E. crassipes* into other tropical and subtropical countries as a decorative plant (Barrett and Forno, 1982). It is widely considered a weed because of its impressive biomass production that restricts water movement and navigation (Gopal, 1987), caused by the high levels of nutrients in the urban wastewater. This species is highly tolerant of a wide range of conditions, including heavily polluted environments, and is successfully used in water treatment systems. The water hyacinth is very effective in extracting excess nutrients (Rogers and Davis, 1972; Wolverton and McDonald, 1979; Trivedy and Pattanshetty, 2002; Jayaweera and Kasturiarachchi, 2004) and heavy metals such as Cd, Pb and Hg (Muramoto and Oki, 1983; Jana, 1987; Cordes et al., 2000; Gardea-Torresdey et al., 2005). Furthermore, a water hyacinth-based phytoremediation system of extended lagoons requires only a small investment in countries where soil prices are low. Moreover, phytoremediation is driven by solar energy and requires little maintenance.

Currently, there is investigation into the parameters of water hyacinth-based water treatment systems that may have a negative effect on the growth and Hg accumulation capacity of this plant (e.g. nutrient level or salinity). This research is expected to provide valuable information about how to improve Hg phytoextraction efficiency and allow water hyacinth to grow in heavily Hg-polluted environments.

In the present study we examined the effect of excess nutrient exposure on the capacity of the water hyacinth for both tolerance and accumulation of Hg. We also assessed the physiological response of this plant to the combined effect of excess nutrients and Hg. A nutrient anion (nitrate) and a nutrient cation (potassium) were supplied in excess. Growth and element content as well as several physiological parameters related to photosynthetic performance were measured to examine the extent of the toxic effects on plants, with a special emphasis on element composition.

MATERIALS AND METHODS

Experimental design: Young water hyacinth (*Eichhornia crassipes* [Mart.] Solms) plants with rosette diameters of approximately 15 cm were purchased from a local nursery (Star Plant, Breda, Spain), washed carefully and placed in 2-L plastic pots containing diluted Hoagland nutritive solution of pH 6.5, the nutrient composition of which was 130.25 mg L^{-1} NO^{3-}, 5.5 mg L^{-1} NH^{4+}, 28.5 mg L^{-1} PO_4^{2-}, 35.5 mg L^{-1} K^+, 24.5 mg L^{-1} Ca^{2+}, 4 mg L^{-1} Mg^{2+}, 14.25 mg L^{-1} SO_4^{2-}, 0.325 mg L^{-1} Fe, 0.240 mg L^{-1} Mn, 0.09 mg L^{-1} Zn, 0.030 mg L^{-1} B, 0.090 mg L^{-1} Cu, 0.028 mg L^{-1} Mo, and 0.005 mg L^{-1} Co.

Plants were grown in a greenhouse in the experimental fields of the University of Barcelona (Spain) in May and June. The average temperature was 18-36ºC, relative humidity was 31-59%, maximum global solar irradiance was 1353 W m^{-2}, and the transmission of the greenhouse covers was 51%. After one week of acclimation, the nutritive solution was amended with potassium nitrate (KNO_3 Panreac, for analysis-ISO), using concentrations of 0, 0.25, 0.5 or 1 g L^{-1} and with mercury chloride ($HgCl_2$ Sigma, minimum 99.5% purity), using concentrations of 0, 2.5, or 5 mg L^{-1}. The resultant ion concentrations are detailed on Table 1. To select the highest concentration of mercury, a preliminary test was conducted with 5, 10 and 25 mg L^{-1} $HgCl_2$, and plants survived only at 5 mg L^{-1}. The experimental design was a two-factor (KNO_3 amendment and $HgCl_2$ amendment) set-up with four and three treatments, respectively. Each treatment was tested on six replicates, grown for eight weeks.

Table 1. Concentration of Hg^{2+}, K^+ and NO_3^- ions (mg L^{-1}) in each treatment.

	$HgCl_2$ (mg L^{-1})			
	0	2.5		5
Hg (mg L^{-1})	0.00	1.84		3.69
	KNO_3 (g L^{-1})			
	0	0.25	0.5	1
K (mg L^{-1})	0.0	0.10	0.19	0.39
NO_3^- (mg L^{-1})	0.0	0.15	0.31	0.61

Chlorophyll content and fluorescence*:* Two days before harvesting the water hyacinths, the chlorophyll content of the base, centre and tip of four mature pre-bloom pseudolaminae was estimated using a portable chlorophyll meter (SPAD-502 Minolta, Illinois, USA), following Krugh et al. (1994). A reading

checker of 72.4±0.3 was used to calibrate the apparatus. Chlorophyll fluorescence was measured with a modulated fluorimeter (Hansatech Fluorescence Monitoring System FMS2, Norfolk, UK) after 30 min of dark adaptation. Maximum quantum yield of PSII photochemistry was calculated as F_v/F_m, given by $(F_m-F_0)/F_m$, where F_m and F_0 were the maximum and initial fluorescence, respectively, in dark-adapted leaves (Nogués et al., 1998). The F_0 was measured for 2.5 seconds with an amber modulating beam (594 nm) at very low intensity (typically 0.001 μmol m^{-2} s^{-1}) and after a saturating pulse of light applied from a Quartz-halogen lamp; the F_m was measured at all wavelengths above 700 nm.

Element determination: Plants were harvested, thoroughly washed, dried in absorbent paper and weighed. Each plant was divided into emerged parts (floats, pseudolaminae and stolons) and submerged parts (roots and rhizomes) in order to record fresh weights and take representative samples of each portion separately. To minimize loss of Hg, samples were immediately frozen in liquid nitrogen, ground with mortar and pestle and stored at -80ºC until analysis. At least 1 g of each sample was dried at 65ºC until constant weight to estimate water content. From each plant, two frozen sub-samples of emerged parts and two of submerged parts, each weighing approximately 200 mg, were digested overnight at 90ºC in a mixture of HNO_3 (Baker, 69.0-70.0%, instra quality) and H_2O_2 (Merck, 30%, suprapur quality) 1:1 vol. Following this, 20 mL Milli-Q deionised water was then added. Digestions were diluted when necessary. Mercury content was determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) performed with a Perkin Elmer apparatus model Elan-6000 (Waltham, Massachusetts, USA), whereas Ca, P, S, Mg, and Mn content were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) using a Perkin Elmer apparatus model Optima-3200RL (Waltham, Massachusetts, USA).

Statistical methods: Multivariate Analyses of Variance (MANOVA) was performed on the basis of a two-factor design with interactions. Logarithmic transformation was used when data did not meet the equal variances assumption. Student-Neumann-Keuls *post-hoc* tests were carried out to assess the differences between groups. All statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 14.0 for Windows. Sigma Plot version 10.0 was used for graphic edition.

RESULTS

Biomass production and photosynthesis: The concentrations of $HgCl_2$ tested did not induce significant changes in *E. crassipes* biomass production (Table 2); however, there was a slight reduction in the fresh and dry weight of the plant, especially in the shoots.

Table 2. Influence of $HgCl_2$ and KNO_3 on the biomass, water content, chlorophyll content and maximum quantum efficiency of PSII of the water hyacinth[a].

		$HgCl_2$ (mg L^{-1})			KNO_3 (g L^{-1})				% SS			
		0.0	2.5	5.0	0.00	0.25	0.50	1.00	$HgCl_2$	KNO_3	KNO_3*$HgCl_2$	Error
FW	Emerged parts	33.4[a]	26.5[a]	26.4[a]	24.6[a]	26.1[a]	35.2[a]	29.1[a]	7.3	2.0	2.2	88.5
	Submerged parts	16.2[a]	11.5[a]	12.9[a]	14.1[a]	12.5[a]	14.9[a]	12.6[a]	6.3	9.4	9.0	75.2
	Total	49.6[a]	38.0[a]	39.2[a]	38.7[a]	38.6[a]	50.1[a]	41.7[a]	7.3	5.9	4.4	82.4
	Submerged/Emerged	0.5[a]	0.5[a]	0.5[a]	0.7[a]	0.5[a]	0.4[a]	0.4[a]	5.7	4.4	1.3	88.7
DW	Emerged parts	2.9[a]	2.3[a]	2.3[a]	2.4[a]	2.2[a]	2.9[a]	2.4[a]	5.0	4.9	6.8	83.2
	Submerged parts	0.9[a]	0.6[a]	0.7[a]	0.9[a]	0.7[a]	0.7[a]	0.7[a]	5.8	3.4	3.8	87.1
	Total	3.7[a]	2.9[a]	3.0[a]	3.3[a]	2.8[a]	3.6[a]	3.1[a]	0.6	8.4	5.3	85.7
	Submerged/Emerged	0.4[a]	0.3[a]	0.3[a]	0.4[a]	0.3[a]	0.2[a]	0.3[a]	0.6	6.8	6.3	86.3
Total FW/DW		13.6[a]	13.4[a]	13.3[a]	12.2[a]	13.6[b]	14.0[b]	13.8[b]	0.4	20.2**	5.9	73.4
SPAD		29.6[b]	21.9[a]	22.5[a]	27.4[b]	26.0[ba]	22.9[a]	22.4[a]	30.1***	10.6**	22.6***	36.7
F_v/F_m		0.832[a]	0.835[a]	0.840[a]	0.856[b]	0.837[ba]	0.823[a]	0.827[a]	0.8	13.4**	29.1***	56.8

[a] Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Means followed by different letters are significantly different by Student-Neuman-Keules with $p>0.05$. Associated percentage of sum of squares (%SS) obtained from multivariate analysis of variance is shown with probabilities * Significant at 0.05 level. ** Significant at 0.01 level. *** Significant at 0.001 level. Fresh weight (FW) and dry weight (DW) are measured in g, chlorophyll content in SPAD units.

The estimated chlorophyll content in green plant parts decreased. In comparison with controls, in which no visible chlorosis was observed, this reduction represented approximately 26% and 24% at 2.5 and 5.0 mg L^{-1} $HgCl_2$ treatment, respectively. The F_v/F_m ratio was the least affected by any of the $HgCl_2$ treatments.

In response to an increasing KNO_3 concentration, there was a significant increase in the water content of plants, expressed by the fresh to dry weight ratio. The greatest difference was found between samples of the control and 0.5 g L^{-1} treatment; however, increasing the KNO_3 supply to 1 g L^{-1} had little effect. The fresh and dry weight of plants tended to be higher when grown at 0.5 g L^{-1} KNO_3, although this pattern was not statistically significant. In plants treated with 0.5 and 1 g L^{-1} KNO_3, average chlorophyll content, expressed in SPAD units, decreased by approximately 16% and 18%, respectively. The F_v/F_m ratio was also reduced due to excess nutrient supply.

The combined effect of $HgCl_2$ and KNO_3 was only significant in F_v/F_m and SPAD; both parameters followed a similar trend (Fig.1). A small reduction in F_v/F_m (Fig.1A) and SPAD (Fig.1B) was induced in plants under 2.5 and 5.0 mg L^{-1} $HgCl_2$ treatment.

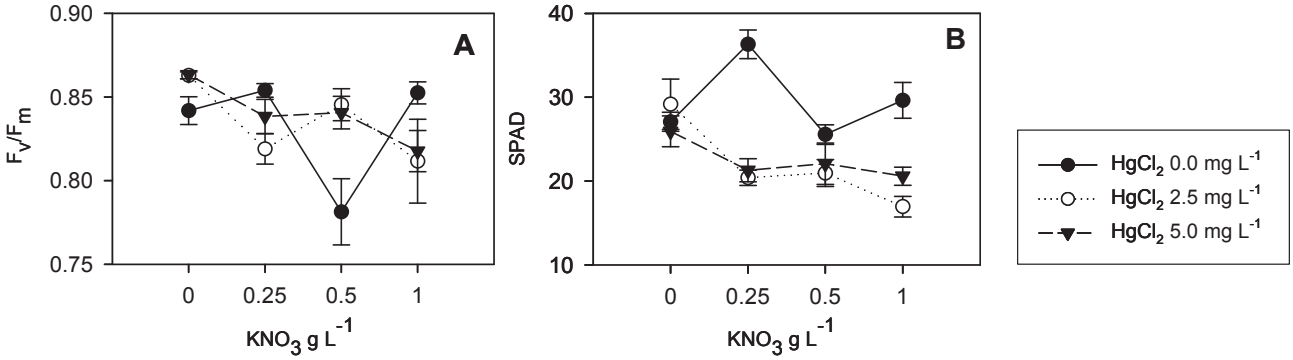

Figure 1. Response of F_v/F_m (A) and SPAD (B) to the combined effect of $HgCl_2$ and KNO_3. Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Error bars indicate standard errors. Each SPAD value is the average of three measurements (base, centre and tip) made on each of n=4 mature pre-bloom pseudolaminae.

Hg uptake: The concentration of Hg increased in tissues of submerged and emerged plant parts as Hg concentration in the growth media was increased (Table 3). Mercury was retained most efficiently in submerged plant parts, reaching 4000 μg g^{-1} of Hg per g of dry tissue at a medium concentration of 5.0 mg L^{-1}, whereas concentrations of Hg in green plant parts were approximately 19-fold less. The ratio of submerged/emerged parts increased when Hg was added to the growth media. This increase was a result of a higher accumulation in submerged parts. However, there was little difference between the 2.5 and 5 mg L^{-1} treatments. The average Hg concentration of the whole plant at the end of the experiment was more than 200-fold higher than the concentration of the growth medium at 5 mg L^{-1} $HgCl_2$ (see Table 1), and more than 175-fold higher at 2.5 mg L^{-1}. Similar results were obtained when the average Hg accumulation per whole plant or plant part was calculated as the concentration multiplied by biomass. Submerged parts extracted a larger amount of the metal, although emerged parts produced more biomass. Changes in KNO_3 concentration had no effect on the content of Hg in submerged parts. However, in emerged sections Hg content decreased with increasing nutrient levels (without attaining statistical significance) and the interaction between both pollutants was significant.

Table 3. Mercury extracted in response to a range of $HgCl_2$ and KNO_3 levels[b].

		$HgCl_2$ (mg L^{-1})			KNO_3 (g L^{-1})				%SS			
		0.0	2.5	5.0	0.00	0.25	0.50	1.00	$HgCl_2$	KNO_3	KNO_3*$HgCl_2$	Error
log Hg concentration	Emerged parts	0.6[a]	1.8[b]	2.2[c]	1.7[a]	1.6[a]	1.5[a]	1.5[a]	84.2***	0.7	2.2	12.8
	Submerged parts	0.1[a]	3.2[b]	3.6[c]	2.3[a]	2.3[a]	2.3[a]	2.3[a]	98.9***	0.0	0.1	1.0
	Total	0.5[a]	2.6[b]	3.0[c]	2.1[b]	2.0[a]	2.1[ab]	2.1[b]	98.1***	0.2*	0.3	1.4
	Submerged/Emerged	0.1[a]	1.4[b]	1.4[b]	0.7[a]	0.9[a]	1.0[a]	1.1[a]	81.6***	1.4	1.0	16.1
log Hg extracted	Emerged parts	1.0[a]	2.1[b]	2.5[c]	2.0[a]	1.8[a]	1.9[a]	1.8[a]	82.6***	0.6	4.3**	12.5
	Submerged parts	0.1[a]	2.9[b]	3.3[c]	2.2[a]	2.1[a]	2.1[a]	2.2[a]	95.7***	0.1	0.0	4.2
	Total	1.0[a]	3.0[b]	3.4[c]	2.5[a]	2.3[a]	2.5[a]	2.5[a]	93.5***	0.5	0.6	5.4
	Submerged/Emerged	0.0[a]	0.9[b]	0.9[b]	0.6[a]	0.5[a]	0.7[a]	0.7[a]	62.2***	1.8	1.8	34.1

[b] Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Means followed by different letters are significantly different by Student-Neuman-Keules with $p>0.05$. Associated percentage of sum of squares (%SS) obtained from multivariate analysis of variance is shown with probabilities * Significant at 0.05 level. ** Significant at 0.01 level. *** Significant at 0.001 level. Log were calculated from Hg concentration in μg g-1 and extracted in μg.

Element content: Plants exposed to Hg showed changes in element composition and distribution (Table 4, Fig.2). With exposure to Hg, P levels in emerged plants declined resulting in a similar trend in both whole plant P concentration and accumulation. The uptake of S per plant also diminished. However, in this case the decrease in concentration was proportional in both plant parts. The distribution pattern of this element was therefore unaffected. The concentration of K in submerged parts decreased and remained unchanged in emerged parts, causing a decrease in the ratio of submerged to emerged parts concentration. As emerged parts account for most of the biomass of the water hyacinth, whole plant K concentration was not affected by Hg treatments. However, there was a decrease in the average accumulation of K per plant. The concentration of Ca and Mg in submerged parts increased in Hg-stressed plants. There were slight differences in the accumulation of these elements among the plants sampled, although no significant difference could be detected. The concentration ratios of Ca, Mg and P in submerged/emerged parts increased at the two Hg concentrations tested. Concerning the Mn concentration, there was a decrease with increasing $HgCl_2$ in emerged parts.

Table 4. Effect of $HgCl_2$ and KNO_3 on element distribution across plant tissues and total accumulation of each element in the whole plant[c].

	$HgCl_2$ treatment in mg L^{-1}			KNO_3 treatment in g L^{-1}				%SS			
	0	2.5	5	0	0.25	0.5	1	$HgCl_2$	KNO_3	KNO_3*$HgCl_2$	Error
Ca											
S/E	1.0[a]	1.2[b]	1.2[ab]	0.8[a]	1.0[b]	1.3[c]	1.4[c]	13.6***	1.7***	10.6**	34.1
TA	37.2[a]	38.9[a]	44.3[a]	44.7[a]	33.6[a]	39.3[a]	42.8[a]	2.3	4.6	4.6	88.5
Mg											
S/E	1.0[a]	1.3[c]	1.1[b]	1.0[a]	1.0[a]	1.2[b]	1.2[b]	4.6***	11.9**	16.9**	46.6
TA	14.8[a]	12.8[a]	12.9[a]	15.5[a]	10.9[a]	13.2[a]	14.4[a]	1.7	6.0	4.6	87.6
K											
S/E	0.7[b]	0.5[a]	0.4[a]	0.4[a]	0.6[b]	0.5[ab]	0.5[ab]	45.0***	9.7**	3.4	41.9
TA	211.3[b]	153.5[a]	160.4[a]	100.1[a]	148.8[a]	235.0[b]	216.3[b]	6.7*	29.4***	7.1	56.7
P											
S/E	0.7[a]	0.9[b]	1.0[b]	0.7[a]	0.8[ab]	0.9[bc]	1.0[c]	20.6***	18.2***	3.9	57.3
TA	23.3[b]	15.0[a]	15.5[a]	20.2	15.5	19.2	17.0	21.1***	4.9	3.3	70.7
S											
S/E	1.7[a]	1.6[a]	1.6[a]	2.0[b]	1.6[a]	1.6[a]	1.5[a]	1.3	9.3***	23.7**	55.7
TA	13.8[b]	9.0[a]	9.8[a]	11.2[a]	9.1[a]	11.4[a]	11.8[a]	16.0**	4.0	3.0	77.0
Fe											
S/E	23.1[a]	29.6[a]	35.1[a]	30.3[a]	23.5[a]	32.4[a]	30.8[a]	6.9	3.4	7.4	82.4
TA	43.5[a]	3.5[a]	4.7[a]	4.9[a]	3.0[a]	4.4[a]	4.5[a]	1.8	3.7	3.4	91.1
Mn											
S/E	7.5[a]	8.6[a]	10.5[a]	7.8[a]	5.0[a]	11.0[a]	11.8[a]	1.5	7.1	17.7*	73.7
TA	0.5[a]	0.4[a]	0.5[a]	0.5[a]	0.3[a]	0.5[a]	0.5[a]	3.0	6.3	2.7	88.0

[c] S/E is the submerged to emerged parts concentration ratio, TA is the total accumulation of each element per plant, given in mg. Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Means followed by different letters are significantly different by Student-Neuman-Keuls with $p>0.05$. Associated percentage of sum of squares (%SS) obtained from multivariate analysis of variance is shown with probabilities * Significant at 0.05 level. ** Significant at 0.01 level. *** Significant at 0.001 level.

An increase of K extraction was observed with greater KNO_3 concentrations, especially in submerged parts. Consequently, the concentration ratio of submerged/emerged parts increased when KNO_3 was added to the nutritive solution, although most of K was retained in emerged parts. Among the elements studied, Ca and Mn exhibited the greatest changes. In submerged parts, the average Ca and P content increased progressively with increasing KNO_3. The resulting concentration of Ca and P was 52% and 19% higher than the controls in the 1g L^{-1} treatment respectively. In emerged parts, Mn concentration decreased by 72% in high KNO_3 treatments and P content by 19%. In contrast, S content was positively correlated with increased KNO_3 and reached a 30% increment at a KNO_3 concentration of 1g L^{-1}. When KNO_3 was added to the nutritive solution, the concentration ratios of submerged/emerged parts increased for Ca, Mg and P; the opposite was true for S. The submerged parts of plants treated with 0.25 and 0.5 g L^{-1} KNO_3 had a lower concentration of Mg and S. The emerged parts of plants treated with 0.25 showed a decrease in Ca and Mg. In terms of the whole plant, the concentration of Ca, Mg and S decreased in plants treated with 0.25 and 0.5 g L^{-1} KNO_3. In contrast, in the 1g L^{-1} treatment the same parameters were lower than controls. The accumulation of Ca, Mg, P, S, and Mn per plant remained unchanged at all the KNO_3 levels tested.

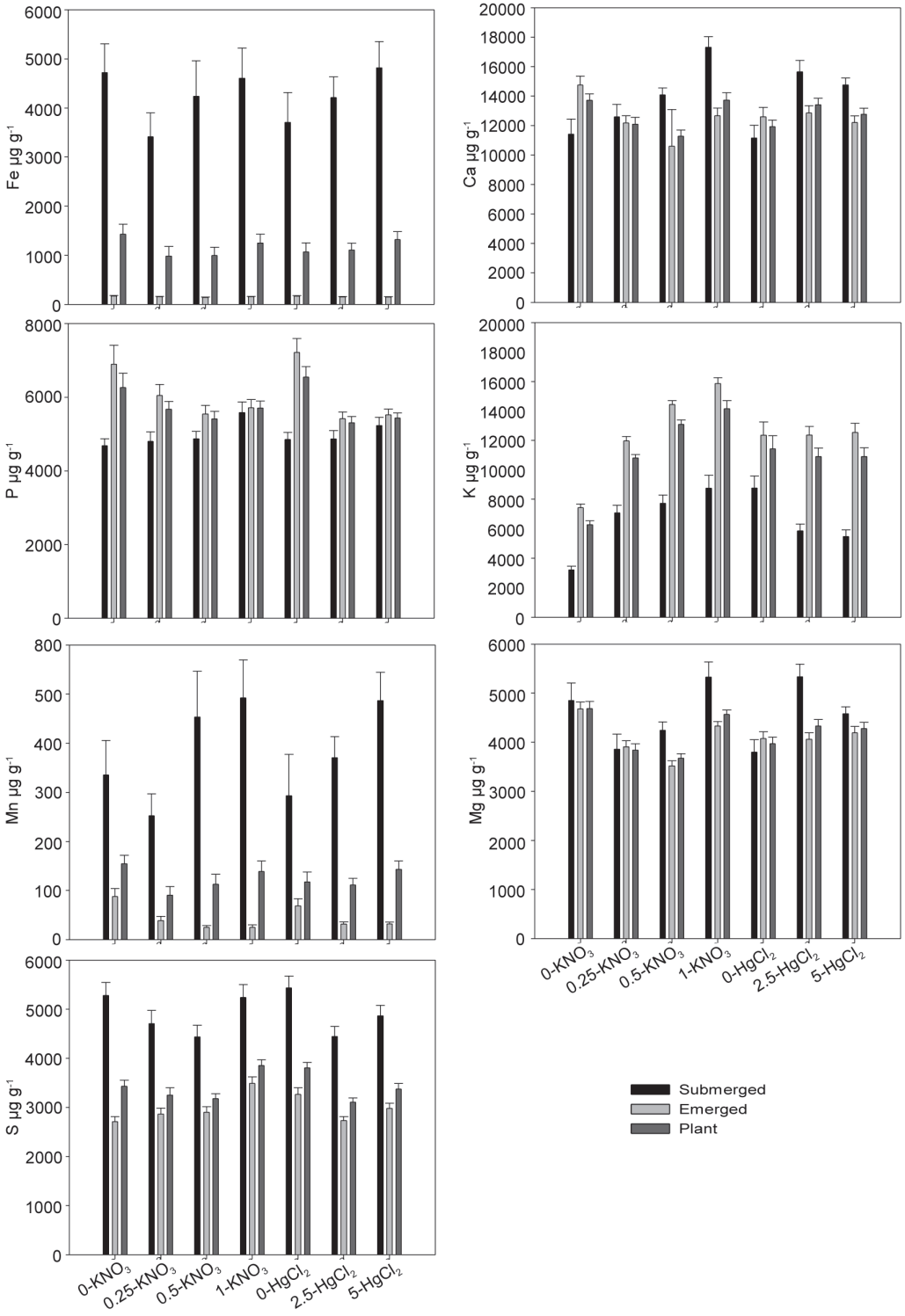

Figure 2. Effect of $HgCl_2$ and KNO_3 on the element composition of plants[e].

[e] Element concentration is given in μg g-1 and element content per plant in mg. Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Error bars indicate standard errors. KNO_3 and $HgCl_2$ treatments are expressed in g L^{-1} and mg L^{-1}, respectively.

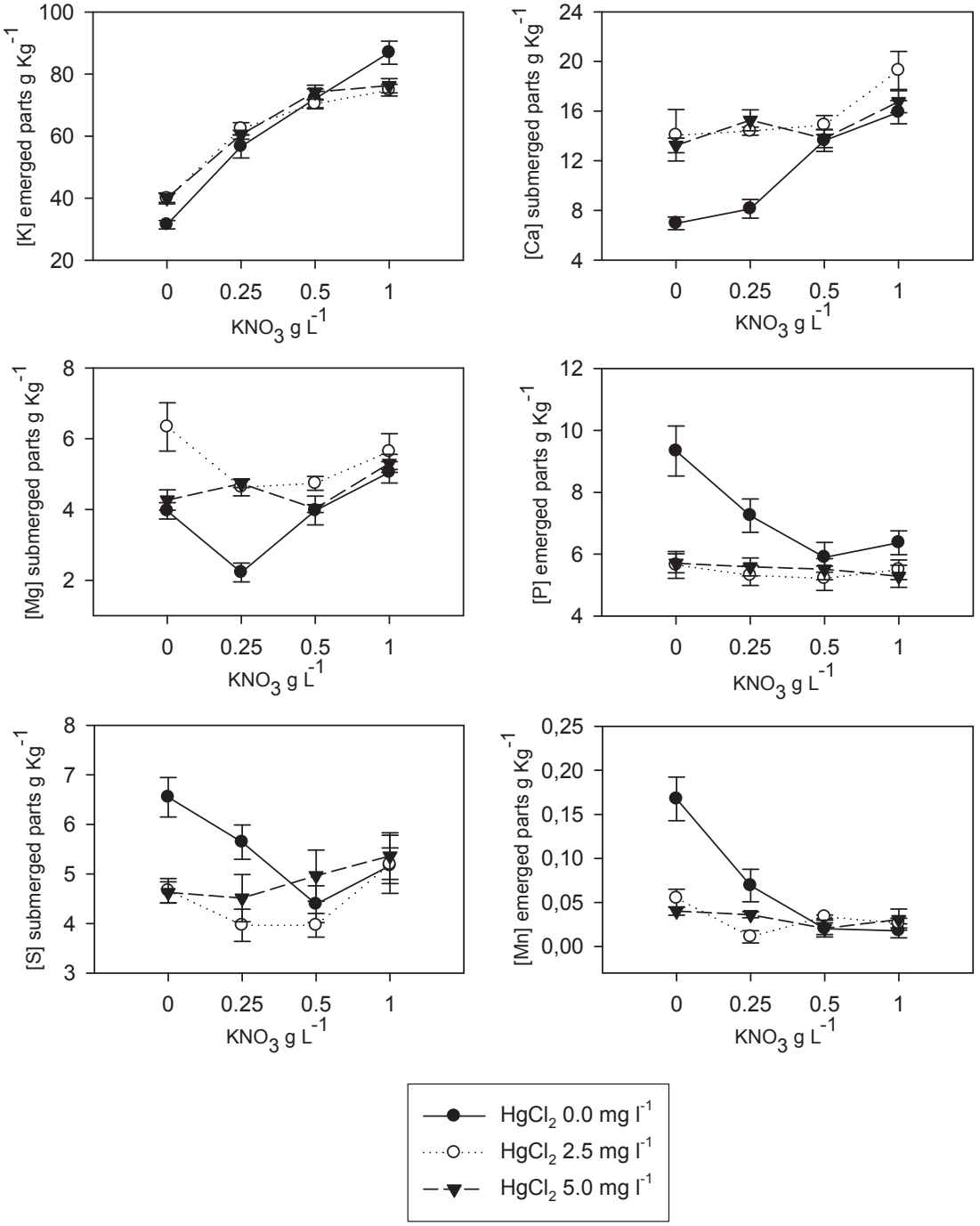

Figure 3. Combined effect of $HgCl_2$ and KNO_3 in element content [f].

[f] Concentration of each element is given in g Kg^{-1}. Values are shown as means (n=24 for $HgCl_2$ treatment and n=18 for KNO_3 treatment). Error bars indicate standard errors. Only the significant interactions according to MANOVA are shown.

The combination of $HgCl_2$ and KNO_3 had significant effects on various different elements. The concentration of K in emerged parts increased with increasing KNO_3, but reached a saturation point at 0.5 g L^{-1} KNO_3 when plants were supplied with $HgCl_2$ (Fig.3). In the submerged parts of plants that were not treated with $HgCl_2$, the concentration of Ca increased dramatically with increasing KNO_3 (Fig.3). Furthermore, in the submerged parts of plants treated with $HgCl_2$ there was a high Ca content even without the addition of KNO_3 and when KNO_3 was increased, Ca content showed only a discrete increment or remained stable. The Mg concentration of submerged parts showed a distinctive pattern for each of the three $HgCl_2$ treatments used at a low KNO_3 supply, but no differences were detected at a concentration of 0.5 g L^{-1} KNO_3.

In the absence of Hg, the initially high concentrations of P and Mn in emerged parts decreased dramatically with increasing KNO_3. The addition of $HgCl_2$ maintained the low and stable levels of both nutrients regardless of the KNO_3 concentration used. The concentration of S in submerged parts followed a fairly similar trend.

DISCUSSION

Effects of $HgCl_2$: E. crassipes exhibited only negligible heavy metal stress symptoms in terms of biomass production, chlorophyll content and maximum quantum yield of PSII, even when $HgCl_2$ was supplied at a high concentration (5 mg L^{-1}) for 8 weeks (Table 2). Chlorosis, reduction of photosynthetic efficiency and retardation of growth have frequently been observed in plants growing in Hg-polluted environments. Mercury affects the biosynthesis of photosynthetic pigments by inhibiting δ-aminolevulinic acid dehydratase (Prasad and Prasad, 1987a, b). The photosynthetic apparatus may also be affected at various levels of PSII, oxygen-evolving complex, PSI, plastocyanin and Ferredoxin/Ferredoxin $NADP^+$ oxidoreductase (Clijsters and Van Assche, 1985; Myśliwa-Kurdziel and Strzałka, 2002; Myśliwa-Kurdziel et al., 2002). All these effects are usually pronounced even at low concentrations of Hg and short exposure times, as several authors have reported for cyanobacteria (Lu et al., 2000), algae (Rai et al., 1991), higher plant seedlings (Prasad and Prasad, 1987a; Munzuroğlu and Geckil, 2002) and submersed aquatic plants (Gupta and Chandra, 1998; Ali et al., 2000), which are less tolerant to the toxic effects of Hg than plants growing on the surface like the water hyacinth (Küpper et al., 1996). Küpper et al. (1996) reported that heavy metals can easily substitute the Mg central atom of chlorophylls, and that Hg-substituted chlorophylls are unstable and unable to emit fluorescence, measured as F_s, the steady-state fluorescence.

However, less attention has been paid to the extent of the toxic effects of Hg on growth, chlorophyll content and the photosynthetic performance of heavy metal-tolerant aquatic macrophytes. Jana (1987) reported no significant reductions in either the growth or chlorophyll content of water hyacinth exposed to a concentration of $HgCl_2$ that was five times lower (1 mg L^{-1}) than that used in our study over a period of 28 days. Consequently, although we detected signs of chlorosis, we found no evidence of growth or F_v/F_m reduction under our experimental conditions.

On the basis of these results, we conclude that photosynthetic apparatus was not affected as a result of a direct action of Hg on chlorophylls, and thus Hg-substituted chlorophylls were not produced. However, further examination of *E. crassipes* physiology under strong Hg stress is required to determine whether the decrease in chlorophyll content is a secondary effect of high Hg accumulation in root tissues or of changes in nutrient composition and distribution rather than the result of the direct interaction of Hg with chlorophyll molecules.

In submerged parts, Hg reached a concentration of about 4000 μg g^{-1} in dry weight, 200-fold higher than that in the growth medium (Tables 1 and 3). As frequently reported in the literature (Beauford et al., 1977; di Toppi et al., 2003; Greger et al., 2005), Hg accumulates mainly in roots; however, the extraordinary Hg-tolerance and accumulation capacity displayed by *E. crassipes* in laboratory trials contrasts with results obtained in field surveys. This discrepancy can probably be attributed to two main factors: (i) the sum of all stress acting in natural environments or constructed wetlands (e.g. plant density, herbivory, nutrient deficiencies, salinity or presence of other pollutants), which can reduce growth and heavy metal tolerance considerably and result in a reduced phytoextraction capacity (Lenka et al., 1990); and (ii) Hg levels are usually much lower in field conditions than in laboratory studies (e.g. Hussain and Jamil, 1990; Riddle et al., 2002), a fact that may be important given that Hg uptake rate depends on external concentration.

Wang et al. (2002) only detected negligible amounts of Hg in water hyacinths cultivated in the laboratory, probably because the concentration of Hg supplied (1.5 μg L^{-1}) was too low. In contrast, Cd added at 8 mg L^{-1} under the same conditions resulted in a Cd concentration of approximately 1000 μg g^{-1} in roots.

A number of authors have reported that the submerged/emerged Hg accumulation ratio increased with an increasing external Hg concentration (Soltan and Rashed, 2003; Göthberg et al., 2004). In our study this proportion showed no significant difference between $HgCl_2$ treatments of 2.5 and 5 mg L^{-1}. The level of Hg detected in the emerged parts of control plants can be attributed either to the proximity of dense traffic or to the reduction or methylation of Hg^{2+}, which may occur in water or in the roots of the water hyacinth (Guimarães et al., 1998). When Hg evaporates from the nutritive solution it can be absorbed by the leaf surfaces of control plants nearby (Göthberg et al., 2004) with little transport to roots. Airborne Hg has been reported to be readily taken up by plant leaves (Mosbæk et al., 1988; Ericksen et al., 2003); the long duration of the present experiment might have allowed a certain degree of such accumulation.

The various concentrations of Hg tested during experimentation caused a reduction in total K, P and S content per plant (Table 4, Fig.2). The K concentration in submerged parts and P concentration in emerged parts decreased. These changes in nutrient uptake or distribution could explain the decrease in chlorophyll content in response to Hg. Godbold and Hütterman (1988) and Godbold (1991) exposed spruce seedlings to 1000 nM $HgCl_2$, and found that in shoots there was reduction of Ca, Zn and Mn, while in roots the same was true for K, Mg, Ca and Mn. Gupta and Chandra (1996) recorded reduced N, P and K content in *Hydrilla verticillata* under Hg stress.

Other trace elements such as Cd and Pb reduce nutrient uptake of plants when applied at toxic concentrations, although the elements and tissues involved and the severity of effects differ between toxic metals and plant species (Walker et al., 1977; Khan and Khan, 1983; Trivedi and Erdei, 1992).

The increase in Ca and Mg content in submerged parts in response to heavy metals could be due to two mechanisms (di Toppi et al., 2003): (i) the adsorption of these elements on the surface of the submerged parts of the plant or (ii) these nutrients are bivalent cations, which may have been retained in roots by the same mechanisms activated by plants in response to heavy metals, probably phytochelatins (Iglesia-Turiño et al., 2006). The increase of Ca in submerged parts exposed to Hg could also be caused by root suberification, which has been reported to occur in response to heavy metals (Barceló and Poschenrieder, 2004). Moreover the increase in submerged/emerged parts ratios for Ca, Mg and P indicates that the transport of these elements to emerged parts was impaired in response to Hg, probably as a tolerance mechanism.

Effects of KNO_3: High levels of nutrients can inhibit plant growth (Marschner, 1995; Wang et al., 2002), but few studies describe the effect of excess NO_3^- or K in *E. crassipes.* Moitra and Pandey (1990) revealed that water hyacinth could tolerate NO_3^- concentrations as high as 3 g L^{-1} in slurry-explosive plant waste waters. Furthermore, higher levels caused plant wilt and severe burns within hours. They recorded no data on plant growth or photosynthetic performance at this concentration, but it is reasonable to assume that these parameters would show a certain degree of alteration before the appearance of such severe symptoms of toxicity. The tendency of growth and photosynthetic inhibition observed in our experiments at high KNO_3 supply can be considered a symptom of incipient toxicity.

The present study also revealed that KNO_3 induced the redistribution of elements within the plant without modifying their uptake. The submerged/emerged parts ratio for K concentration increased in parallel with external supply, indicating that submerged parts have a proportionally greater capacity to increase their K content. Shiralipour et al., (1981) found that as N concentration increased in 10% Hoagland, P uptake increased or decreased depending on P levels in the nutritive solution. Under the conditions of the present study, no significant changes in P content were observed. This absence of variation could be a result of the balance between N and K maintained in all the treatments; however, further studies are required to verify the relationship between supply and uptake of these three macronutrients in *E. crassipes*. In conclusion, these observations indicate that KNO_3 added to nutritive solution at concentrations higher than 0.5 g L^{-1} could be toxic to the water hyacinth.

Interaction between $HgCl_2$ and KNO_3: Heavy metal uptake by plants may increase, decrease or remain stable at a range of nutrient levels depending on several mechanisms;

these include the competition between nutrient cation and heavy metals for uptake sites, limitation of bioavailability by nutrient anion complexation with heavy metals, and plant growth enhancement promoted by high nutrient levels. The assessment of the interaction mechanisms between heavy metal and cations is of great relevance to solve the limitations of phytoremediation in multipolluted sites, but also to provide with useful data for the effective control of *E. crassipes* expansion. Göthberg et al. (2004) examined the effect of nutrient levels on Hg, Cd and Pb uptake in water spinach and concluded that Hg concentration in roots was not influenced by the degree of nutrient dilution in the external medium, while Hg concentration in shoots increased with decreasing nutrient levels. The results of the present study revealed a very similar pattern in the water hyacinth though with higher Hg^{2+} concentrations (3.69 ppm vs. 0.05 ppm). However, this is of little relevance for Hg removal since emerged parts make only a small contribution to the total amount of Hg extracted by the plant.

The addition of other cations can lead to a reduction of heavy metal uptake and consequently to a higher tolerance. Sepehr and Ghorbanli (2006) observed that Cd and NaCl at moderate levels reduced the individual toxic effects of both stresses on *Zea mays* seedling growth and chlorophyll content. This was because NaCl inhibited Cd influx. In the present work, plants showed no amelioration of stress because Hg uptake was not significantly affected by KNO_3 (Table 3). Conversely, the capacity of the water hyacinth to extract excess K from polluted waters was diminished by approximately 24% at 5 mg L^{-1} $HgCl_2$ (Table 4).

CONCLUSIONS

In this study, a weak competition for the uptake of both pollutants, and a small synergistic effect on chlorophyll content and fluorescence were observed. This poses only a minor inconvenience for phytoremediation, as *E. crassipes* growth is hardly affected, and Hg uptake is maintained. Mercury is far more toxic and expensive to remove from water than KNO_3, and is therefore a priority. We conclude that *E. crassipes* has a strong capacity to withstand and extract Hg, and that this capacity is not impaired by excess nutrient supply. Therefore this species is a promising candidate for Hg remediation of eutrophic waters containing high levels of K and NO_3^-. However, further research is required to assess the performance of *E. crassipes* under field conditions.

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