General and Applied Plant Physiology – 2009, Volume 35 (3–4), pp. 179–187

Available online at http://www.bio21.bas.bg/ipp/

Special Issue (Part I) – Proceedings of the XI National Conference on Plant Physiology
18–19 November 2009, Sofia, Bulgaria

COPPER ACCUMULATION CAPACITY OF TAMARISK (*TAMARIX TETRANDRA* L.) AND WHITE MULBERRY (*MORUS ALBA* L.) DEPENDING ON SOIL TYPE

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Received: *20 January 2010* Accepted: *22 February 2010*

Summary. Saplings of tamarisk (*Tamarix tetrandra* L.) and mulberry (*Morus alba* L.) were grown on contaminated with 120 ppm Cu two types of soil - alluvial-meadow soil (pH 6.5) and cinnamon-red forest soil (pH 5.2) with the aim to evaluate plant Cu accumulation capacity. The growth of plants on alluvial-meadow soil was not affected by high Cu content in contrast to plant reaction on cinnamon-red forest soil. Analysis of Cu in plants after 90 days of growth reveals that tamarisk can be regarded as Cu extruder while mulberry showed the properties of Cu accumulator. Plants Cu tolerance is explained through the mechanism of regulation of plant water relations or with the ability of plants to accumulate increased amounts of organic acids in the fine roots.

***Key words*:** tamarisk, mulberry, Cu accumulation, soil type, transpiration, bound organic acids.

***Abbreviations*:** Cu – copper ions, WUE – water-use efficiency, OA – organic acids.

INTRODUCTION

One promising way to ameliorate harmful effects of metal toxicity is to cultivate plants with high metal accumulating capacity (Baker, 1981; Prasad and Hagemayer, 1999; Ernst et al, 1992). These plants known as metal hyper accumulators can be grown successfully in urban area with polluted soils. Among the group of plants recommended for plantation in such areas perennials are more efficient because of their larger remediation capacity (Fernandez and Henriques, 1991; Wang and Chen, 2009). Although more efficient as ornamentals and metal extractors their growth tolerance metal polluted soils is largely unknown. First of all, successful rooting of tree saplings or shrubs transplanted in any polluted soils

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depends on plant physiological tolerance to particular metal contaminant, soil acidity, moisture capacity and nutrient availability during growth season (Fernandez and Henriques, 1991). High soil acidity can increase solubility of harmful metal ions such as Al or Mn (Salt et al., 1995; Baker, 1981). Higher or low moisture of soils also influence metal accumulating capacity (Fernandez and Henriques, 1991; Foy et al., 1978). Temperature, wind and light are factors that can affect plant metal toxicity tolerance (Fernandez and Henriques, 1991). The most important among factors influencing plant remediation capacity are physiological and genetic factors determining species capacity to take harmful ions or to exclude them sustaining growth with suitable rate. Tamarisk, salt cedar (*Tamarix tetrandra* L.) is one of 55 species of the family *Tamaricaceae,* graceful hardy shrub, native in Mediterranean and Central Asia, developing nice pale pink flowers during growth season and grown well in urban area as ornamental plant. It is propagated by stem cuttings, grows well in poor sandy soils and shows some drought tolerance. However, the ability of this species to tolerate high concentration of metal pollutants in soil is largely unknown. The other plant species white mulberry or morus (*Morus alba* L.) is deciduous tree, originated from East Asia and planted broadly in urban and agricultural areas. Its taxonomy is complex as it has been selected through hybrid technology. It grows well on poor and drought soils. The characteristics of these species inspire us to study some physiological responses of plant saplings to soils contaminated with Cu.

The object was to study plant dry matter accumulation, uptake and storage of copper ions in different organs and water-use efficiency during vegetative season related to copper accumulation. All experiments were carried out in a green house with plants grown in pots filled with two types of soil differing in their nutritional capacity and acidity.

MATERIALS AND METHODS

Saplings of tamarisk (*Tamarix tetrandra* L.) and mulberry (*Morus alba* L.) were propagated from stem cuttings in sand and then transferred to plastic pots. Every pot contained one sapling planted in 12 kg of dry soil. Two type of soil were used in the experiment. One set of 12 pots were filled with alluvial-meadow soil (G. Lozen Institute of Plant Physiology Experimental Field) and the other set was loaded with cinnamon-red forest soil (G. Banya Experimental Field) (Table 1). Soil samples were analyzed for N and P and K (Bartels, 1996). In the other set of 6 pots 0.1 mM solution of $CuSO_4.5H_2O$ was added to final concentration of 120 ppm Cu in the soil. Soil moisture in the pots was kept at 70% of the soil full moisture capacity during the time of experiment. After 90 days of growth plants were harvested, separated into roots (fine plant roots <1-2 mm and coarse roots >1 mm), stems, leaves then weighted, dried and milled into fine powder <0.04 mm. Aliquots of milled plant material were dry ashed at 550°C. Ashes were dissolved in 4 ml hot 20% HCL, transferred to 50 ml flask and diluted with distilled water. This solution was analyzed for Cu with atomic absorption spectrometer Karl Zeis (Germany). Total organic acids (OA) in plant tissues were determined with the

Table 1. Characteristics of different types of soils used in the pot experiment

Soil types	Total hydrolysable N, mg/100 g dry soil	Total P, mgP_2O_5/100g dry soil	Total K, mg K_2O/100g dry soil	pH of soil solution in water	Total organic matter,%	Exchangeable Al_2O_3, meq/100g
Alluvial-meadow soil	4.06	4.90	18.0	6.5	1.4	0.02
Cinnamon-red forest soil	4.20	1.52	31.7	5.2	1.1	0.62

method described by Pochinok (1976). OA were extracted with acidified 70% ethanol and precipitated with $Pb(CH_3COO)_2$ .The pellet were dissolved in 1% Na_2CO_3 and obtained soluble Pb, in equivalent of OA in solution, was determined by titration procedure (Pochinok, 1976). The quantity of transpired water per plant for the growth season was calculated by subtracting the soil evaporation from the total evapotranspiration. Sum of daily transpired water for growth season of one plant was used for calculation of transpiration efficiency. Analysis of variance was conducted to determine any significant differences among the parameters between treatments and control. The factors with statistical significant main effects were analyzed with Duncan's multiple range tests. Program Stat graphics plus, version 2.1was used (Statistical graphics Corp. USA).

RESULTS AND DISCUSSION

Tamarisk saplings grown on contaminated alluvial soil increased relative value of dry mass of fine roots<1 mm in comparison with other plant organs (Fig 1).

Mulberry saplings also developed better root system on the alluvial- meadow soil but grew well on the cinnamon–red soil. The mulberry saplings planted on the contaminated cinnamon red forest soil showed better growth than tamarisk. Worse growth of both plant species on cinnamon-red soil could be associated with poor fertility of this soil type. Lower pH of soil solution can increase the solubility of harmful ions which can also contribute for the inhibition of plant growth. Dry matter accumulation of plants closely related to the accumulation of Cu ions among plant organs (Table 2). Tamarisk accumulated almost 85% of Cu in the roots but nevertheless it behaved like poor Cu accumulator on both contaminated soils (accumulated Cu ranged between 40-100% of control). The distribution of accumulated Cu in mulberry differed greatly from that of tamarisk. Mulberry could be characterized as better Cu accumulator when grown on the Cu

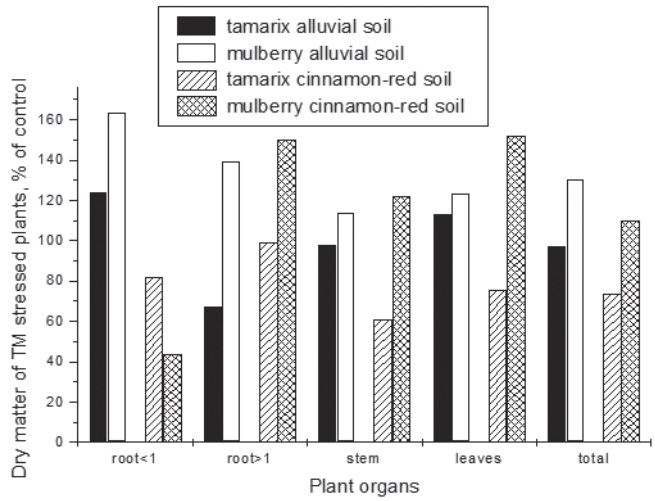

Fig.1. Organ distribution of dry matter of tamarisk and mulberry saplings (% of control) per plants grown in contaminated with 120 ppm Cu alluvial – meadow and cinnamon-red forest soil.

Table 2. Accumulation of Cu in tamarisk (*Tamarix tetrandra* L.) and mulberry (*Morus alba* L.) grown on 2 types of soil contaminated with 120 ppm Cu.

Plant Species	Plant organ	Alluvial-meadow soil					Cinnamon-red forest soil				
		Concentration [mg Cu/100g DW]		Accumulation [µg Cu/plant]			Concentration [mg Cu/100g DW]		Accumulation [µg Cu/plant]		
		Control	Stress	Control	Stress	% of Control	Control	Stress	Control	Stress	% of Control
Tamarisk	r<1	1.42	3.72*	19	63*	331	0.05	2.95*	0.4	20*	5000
	r>1	2.35	2.92**	178	150**	84	0.10	0.23*	3	7**	225
	stem	1.55	0.22*	260	36*	14	3.35	2.75*	282	133*	47
	leaf	1.15	0.04*	146	5*	4	0.85	3.22*	67	194*	289
	plant	-	-	603	253*	42	-	-	352	354**	101
Mulberry	r<1	3.92	2.90**	410	420	103	0.01	7.2*	1	392*	39200
	r>1	0.12	0.50*	20	180	900	1.40	1.0*	87	93**	106
	stem	0.10	0.95*	40	424	1050	0.40	0.4**	49	59*	120
	leaf	0.10	0.60*	26	194	950	0.22	0.2**	20	31*	155
	plant	-	-	496	1218	246	-	-	157	575*	366

Data are means of 3 replicates; * significant or ** not significant from control (P≤0.05)

polluted soils. Saplings contained 245.6% of control on contaminated alluvial meadow soil and 366% when grown on polluted cinnamon-red soil. Most of the taken up Cu was located in the fine roots and stems.

Table 3 presents results on the effect of Cu contamination on the plants water relations. Both species accumulated more dry matter on polluted soils but evaporated less water per plant for the season. This effect was more pronounced in plants grown on cinnamon red forest soil. Due to the better plant growth on polluted alluvial meadow soil plant water-use efficiency for growth season was higher for both plant species. In contrast, plants grown on polluted cinnamon- red forest soil, despite of inhibited transpiration rate and total evaporated water per plant, did not change significantly their WUE for the season.

If the quantity of water for accumulation of one unit of Cu per plant was calculated, it could be seen that tamarisk spent less water for accumulation of unit of Cu per plant in comparison with mulberry saplings (Fig. 2). However, on contaminated soils tamarisk used more water for Cu uptake than mulberry which transpired significantly less water for accumulation of unit copper per plant. At the same time mulberry transpired more water per season for the accumulation of Cu under control conditions but strongly reduced this parameter when grown on the contaminated soils. Regarding the fact that it was more efficient as Cu accumulator it can be concluded that mulberries control their water relation on polluted with Cu soils more efficiently.

Table 4 presents results for total quantity of bound organic acids (OA) in the fine roots of plants (root <1 mm) grown on two types of soils. Fine roots were chosen for the analysis as most of the accumulated Cu in both species was found to be located in this plant organ. The quantity of bound OA precisely related to the concentration of Cu accumulated in the roots of plants grown on polluted soils. One exception are the results for fine roots of mulberry grown on polluted alluvial-meadow soil. This is not surprising since most of Cu was found in the stem, but not in the roots of plants (Table2).

As the metal toxic tolerance of plants

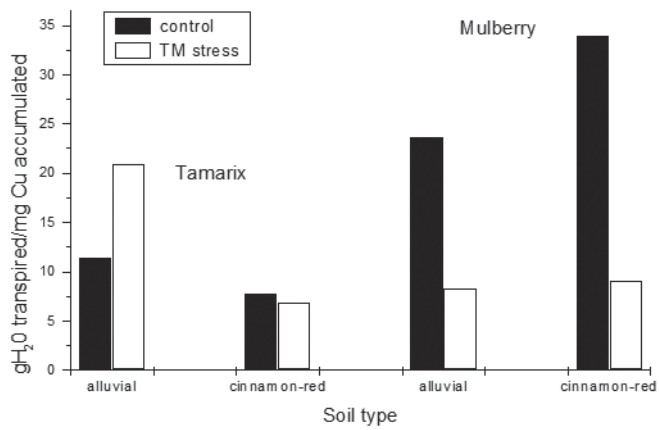

Fig.2. Relationship between total transpiration per plant and uptake and accumulation of Cu in control and stressed plants grown on different types of soil.

Table 3. Transpiration rate and water –use efficiency for whole growth season (90 days) of tamarisk and mulberry saplings grown on soils contaminated with Cu.

Plant species	Treatments	Alluvial-meadow soil				Cinnamon-red forest soil			
		Plant dry matter [g/plant]	Total transpired water per plant during growth season [g H_2O/plant]	Mean transpiration rate of plant [g H_2O/g Dwt/day]	Water-use efficiency of plants [g DW/g water]	Plant dry matter [g/plant]	Total transpired water per plant during growth season [g H_2O/plant]	Mean transpiration rate of plant [g H_2O/g Dwt/day]	Water-use efficiency of plants [g DW/g water]
Tamarix	Control	38.48	6872	1.99	5.61	20.00	2755	1.53	7.29
	Stress	76.13*	5290**	0.76*	14.49*	14.72*	2440**	1.85**	6.02*
Mulberry	Control	98.55	11795	1.32	8.40	45.50	5337	1.30	8.52
	Stress	128.5*	10150**	0.87*	12.60*	41.10**	5267**	1.42**	7.56*

Data are means of 6 replicates; * significant or ** not significant from control ($P \leq 0.05$)

Table 4. Relationship between accumulated Cu and total bound organic acids determined in the fine roots <1 mm of tamarisk and mulberry saplings grown on control and contaminated soils.

Treatments	Cu concentration [μg/g Dwt]	% of control	Total bound OA[a] concentration [mg/g Dwt]	% of control
Alluvial-meadow soil				
Tamarix control	14.2	100.0	3.54	100.0
Tamarix TM[b] stress	37.2*	261.9	4.63*	130.7
Cinnamon-red forest soil				
Tamarix control	5.0	100.0	3.33	100.0
Tamarix TM stress	29.5*	590.0	4.67*	141.0
Alluvial-meadow soil				
Mulberry control	39.2	100.0	2.63	100.0
Mulberry TM stress	28.0*	71.4	2.20**	93.2
Cinnamon-red forest soil				
Mulberry control	10.0	100.0	1.23	100.0
Mulberry TM stress	72.0*	720.0	2.59*	210.5

Data are means of 3 replicates; * significant or ** not significant from control (P≤0.05).
[a]- total bound organic acids, mg malate/g plant DW.
[b]- toxic metal stress - 120 ppm Cu in soil.

depends on the ability to sustain growth in polluted soils or to detoxify or exclude these toxic ions form plant tissues we can conclude that both plant species can be successfully used for planting in soils polluted with Cu. However, the mechanisms of Cu toxic metal tolerance of plants differ significantly. Tamarisk controls uptake of Cu by decreasing the plant water-use efficiency while the mulberry regarded as Cu accumulator better control its water relations which allows it to grow more successfully on both soil types. Barcelo et al. (1986) also discussed the effect of toxic Cd applied to the roots of bushy bean plants on plants water stress resistance. Accumulation of Cu in plant tissue caused oxidative stress which activated the antioxidant system of plants (Doncheva et al., 2006; Teisseire et al., 1998). The soil type influences the ability of species to sustain higher internal Cu by affecting their nutrient-use efficiency (Xia and Chen, 2007). Analysis of results revealed that addition of exogenous succinate can affect the mechanisms of toxic Cu tolerance (Doncheva et al., 2006). Tukendorf and Baszynski (1985) and Drazic et al. (2006) have reported that different proteins can be synthesized as a response to Cu influence on plant metabolism. Although direct correlation between amount of total organic acids in the fine roots and accumulated Cu in this organs was found, more detailed studies on the metabolic response of plants to Cu stress should be done to reveal the basic mechanism of the tolerance (Demirevska- Kepova et al., 2004).

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