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APPLICABILITY OF Cu AND Zn HYDROXOSALTS AS SOURCES OF MICROELEMENTS FOR GROWING LETTUCE (*Lactuca sativa* L.). I. METHODOLOGICAL APPROACH

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Summary. Copper and zinc are vitally important nutrient elements, but are also potential soil polluters. More promising forms have to be found, in which these microelements could be presented to the plants. The aim of this investigation was to modify the method of water cultures in such a way that the concentration of copper and zinc hydroxosalts could be controlled in order that their qualities as sources of microelements could be compared to the simple salts of sulphur acid.

Vegetation pot experiments were conducted with the synthesized hydroxyde carbonates and hydroxyde sulphates of Cu and Zn in water cultures using the lettuce cv. Zhulta Gyumyurdzhinska. The hydroxosalts of Cu and Zn are equivalent sources of microelements and as compared to simple salts stimulate the root's growth. Hydroxyde carbonates are better as a source of Zn and hydroxyde sulphates – as a source of copper.

Key words: hydroxosalts, sources of microelements, copper, zinc, water cultures

Introduction

Copper and zinc are vitally important nutrient elements, but are also potential soil polluters. Therefore the question for finding more promising forms arises in which these microelements must be presented to the plants.

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Usually as sources of Cu and Zn for the plants are used their salts with H_2SO_4 or HCl (Stanchev et al., 1982). They are water soluble and are applied for presowing treatment of the seeds and for extrarooted fertilizer application. Phosphorus mineral fertilizers are a considerable source of Zn pollution in agriculture.There Zn content varies from 50 to 1450 ppm, while the Cu content is found mainly in plant protecting chemicals.

In the opinion of Mirchev (1971) the average Zn content in Bulgarian soils is 75 ppm. Despite the presence of Cu and Zn at pH above 7 in the chemicals (preparations), they pass into insoluble hydroxides and become less accessible for plants.

If we use the analogy with phosphorus in investigating the interaction between the form of phosphates in the soil and their accessibility to plants (Mattingly, 1965; Garbuchev, 1978) we should expect that the presence of difficult, moderately and easily soluble compounds in the nutrient substratum could ensure a constant supplement of soil solution with accessible to plants Cu and Zn. Therefore the problem arising consists in a necessity to search for new compounds with intermediary solubility between chlorides, sulphates and hydrooxides. Hydroxosalts are in agreement with this condition. Some crystal chemical characteristics and there low solubility allow us to expect that these compounds would be suitable sources of Zn and Cu microelements. They are characterised with low toxicity and are acceptable for plants.

The aim of the present investigation was to modify the method of water cultures in such a way that the concentration of hydroxosalts of copper and zinc can be controlled in view of comparing their qualities (characteristics) as sources of microelements with the simple salts of sulphuric acid.

Material and Methods

Pure hydroxosalts (carbonates and sulphates) were produced after the method of continuous precipitation from sulphate solutions of copper and zinc with Na_2CO_3 and NaOH. The solid phases precipitated were structural compounds of the salts:
$Cu_2(OH)_2CO_3$; $Zn_5(CO_3)_2(OH)_6$; $Cu_4(OH)_6SO_4$; $Cu_3(OH)_4SO_4$; $Zn_4(OH)_6SO_4.5H_2O$.

Vegetation experiments were conducted with the synthesized compounds using the widely distributed lettuce cv. Zhulta gyumyurdzhinska grown as a water culture. Disinfected germinating seeds were sown in balkanine. They were sprayed until germination with temperate tap water, and after the cotyledons appeared – with 0.1 FNS. The plants were transferred to the experimental solution at the phase appearance of second true leaf. The nutrient solutions (FNS) used, intended especially for lettuce (Georgieva and Nicolova, personal communication) had the following composition: 2.45 mM $Ca(CO_3)_2.4H_2O$; 1.33 mM $KHPO_4$; 2.08 mM KNO_3; 3.5 mM $MgSO_4.7H_2O$ and 0.7 mM KCl. Microelements from A–Z (ME) were introduced after Hoagland. Copper and zinc sulphates were excluded and added individually. Iron was supplemented as Fe-EDTA in a concentration of 0.089 mM.

The experiment was carried out after the following scheme:

1. FNS + $CuSO_4$ + $ZnSO_4$ + Fe-EDTA + ME
2. Zn-hydroxide carbonate + FNS + $CuSO_4$ + Fe-EDTA + ME
3. Zn-hydroxyde sulphate + FNS + $CuSO_4$ + Fe-EDTA + ME
4. Cu-hydroxyde sulphate + FNS + $ZnSO_4$ + Fe-EDTA + ME
5. Cu-hydroxyde carbonate + FNS + $ZnSO_4$ + Fe-EDTA + ME

The hydroxosalts investigated were applied once at the time of planting in the vegetation pots. Hydroxosalts concentration was calculated on the basis of the necessary quantity of zinc for the entire growth period. The plants were grown after a modified method of supplementation (Ingestao, 1970, 1971). Ion concentration in the nutrient medium was followed conductometrically and was corrected by applying supplements of FNS, ME and Fe-EDTA. The nutrient medium was aerated. The concentration of copper, zinc and iron cations in the nutrient medium was determined twice during the growth period (on the 3rd day and at the end of the experiment), on an atomic absorption spectrophotometer. The concentration of hydrogen cations in the solutions was measured at the time they were prepared and 12 days after the experiment began. Plants were harvested at the phase of technical maturity and their measurements were taken. From fresh leaf material were determined: in extract with 2% oxalic acid vitamin C – titrametrically with dichlorophenolindophenol; carotenoides in 80% acetone extract – spectrophotometrically after the method of Arnon. Free nitrate content was determined from dry material with 5% salicylic acid, after the method of Cataldo (1975). Total nitrogen and phosphorus content was determined after wet burning with concentrated H_2SO_4 and H_2O_2. Nitrogen was determined as asmerald green on the Kontiflo apparatus, phosphorus – colorimetrically after Kozhuharov (1960) as molybdenum blue with a double reducer hydrazine sulphate and stanochloride. Ca, Mg, Cu, Zn and Fe were determined after dry ashing and solving with 20% HCl on an atomic absorption spectrophotometer (Stanchev et al., 1982).

Results and Discussion

Data presented in Table 1 show that on the third day after copper hydroxosalts are 35–37% dissociated, while the zinc ones are characterized by a higher degree dissociation – 48–58 %. This difference in their reactions is conserved until the end of the growth period and for each salt respectively the dissociation increases approximately 20%. According to the analysis 40–46% copper salts and 24–30% of the zinc salts are preserved in an undissolved state.

Analysis of nutrient solutions during the growth period leads to the conclusion that in the NO_3^- source of nitrogen used and the effect of the plants with time the solutions are alkalized. From a starting pH of the solution 5.6 the equilibrity for both

Table 1. Characterization of the nutrient medium (mg/l)

Treatments	Concentration at the beginning		Duration (days)						pH
			3	17	3	17	3	17	
	Cu	Zn	Cu		Zn		Fe		
Control	0.014	0.022	0.020	0.230	0.022	0.440	2.340	2.070	6.79
Zn hydroxyde carbonate	0.014	5.320	traces	0.140	3.080	3.740	1.910	–	6.86
Zn hydroxyde sulphate	0.014	5.320	traces	0.180	2.530	4.070	2.430	0.790	6.70
Cu hydroxide sulphate	3.560	0.022	1.310	2.140	0.190	0.320	–	0.890	6.94
Cu hydroxide carbonate	3.560	0.022	1.250	1.940	0.380	0.520	2.180	1.190	6.50

Dissociation of Cu and Zn in the nutrient solution

	after 3 days	after 17 days
Cu hydroxide sulphate	37%	60%
Cu hydroxide carbonate	35%	54%
Zn hydroxide sulphate	58%	76%
Zn hydroxide carbonate	48%	70%

Table 2. Biomass accumulated in lettuce at the phase of harvesting, expressed in g/1 plant and biometrics indices

Treatment	Biomass	Leaves	Stems	Roots	Whole plant	Length (cm) Leaves	Length (cm) Roots	Leaves Number
Control	FW*	32.255±2.888	1.977±0.253	9.372±1.037	43.604±2.222	14.8	8.2	15.0
	DW*	3.505	0.358	0.664	4.527			
Zn hydroxide carbonate	FW	37.416±3.366	2.222±0.315	9.950±0.893	49.588±2.732	15.3	7.8	19.5
	DW	2.925	0.401	0.771	4.092			
Zn hydroxide sulphate	FW	36.127±4.068	2.362±0.308	9.278±1.563	47.767±3.396	15.3	8.7	17.3
	DW	3.015	0.455	1.158	4.655			
Cu hydroxide sulphate	FW	37.115±2.244	1.883±0.180	10.835±1.044	49.833±1.904	17.2	10.2	15.8
	DW	5.805	0.395	1.126	7.385			
Cu hydroxide carbonate	FW	31.064±4.253	1.940±0.452	9.776±1.341	42.780±2.989	16.4	7.6	15.4
	DW	4.929	0.420	0.935	6.284			

FW* – fresh weight; DW* – dry weight

kinds of salts establishes itself at 6.5–6.86 or these values correspond to a pH of neutral soil from pH 6.5–7.5 (Ganev,1990).

Table 2 presents the data about biometrics measurements of plants at the phase of technical maturity. A trend is observed toward better plant development in case zinc is available as basal carbonate, while Cu as basal sulphate. In case hydroxosalts are used as sources of copper and zinc, there exists a trend towards increasing the share of the roots. This result is important because lettuce is a crop with small seeds, short growth period and slow growth of the roots in the first 14 days (Costigan, 1987).

Some authors (Costigan, 1987; Tyksinski, 1992) state that crops with small seeds are with very high requirements for the nutrient regime in the first 14 days and also have greater requirements toward the concentration of nutrient elements in the zone around the roots.

There are no statistically significant differences in fresh biomass between variants. Therefore a conclusion can be drawn that hydroxosalts are not inferior to salts as sources of microelements. Taking into account that a part of the salts are stored in a not disturbed state, this would guard the soil solution and the surface waters from pollution. They have an advantage as compared to simple salts the viewpoint of preserving the environment.

Data about microelements contents of Cu, Zn and Fe in the organs of lettuce could be discussed from several aspects (Table 3). No significant deviations in the contents of macroelements N, Ca and Mg were observed in the three sources of copper and zinc. The trend toward increasing P content parallel to the risen Zn concentration in the nutrient medium should be noticed. Similar results concerning the relation phosphorus–zinc have been observed in maize (Stoyanova, 1988), wheat (Jianqun et al., 1992) etc. Cooper and zinc content in general lines follows their concentration in the nutrient medium, there content in the leaves ranging in smaller limits, while the surplus amounts are blocked first in the roots followed by the stems.

Iron content in the organs of lettuce depends on the sources of copper and zinc. In case hydroxosalts are used, the content of iron in the leaves is reduced, but increases in roots and stems. This process is more obvious in case carbonates are used. As known, zinc phytotoxicity is blocked by application of calcium carbonate, but up to a definite concentration of the polluter (Lyszcz, 1991) and for the three elements their elements in the leaves are within the norm (Bergmann at al., 1976; Bergmann, 1988). Data in Table 4 concerning the biochemical characterization of lettuce leaves in market ripeness indicate that the quantity of free nitrates is low and between simple salts and hydroxosalts no significant differences are observed. The same could be said for vitamin C and carotenoids. Data concerning the content of copper and zinc expressed as mg/1000 g of fresh leaf weight do not surpass the limits indicated by the Bulgarian State Standard. Arrambarri et al. (1987) used hydroxide chlorides copper, zinc and cobalt and composed them in a sand culture with simple chlorides and their hydroxides. They reached the conclusion, just as we did, that if the concentrations are properly chosen, the basal salts are of equal value with the simple ones as sources of the respective microelements.

Table 3. Chemical composition of lettuce in the phase of harvest maturity

Treatment	Organ	% of dry weight				mg/1000 g dry weight		
		N	P	Ca	Mg	Cu	Zn	Fe
Control	leaves	1.88	0.71	0.44	0.28	6.88	80.00	213.12
	stems	0.87	0.36	0.31	0.10	12.50	200.00	288.75
	roots	1.11	1.08	0.54	0.22	25.62	307.50	857.50
	whole plant	1.68	0.80	0.40	0.26	10.07	122.08	313.66
Zn hydroxide carbonate	leaves	1.92	1.18	0.51	0.28	13.75	160.00	82.50
	stems	0.90	0.80	0.28	0.10	21.25	180.00	563.75
	roots	1.45	1.00	0.61	0.41	77.50	155.50	1993.80
	whole plant	1.73	1.04	0.50	0.29	26.50	349.50	489.77
Zn hydroxide sulphate	leaves	1.68	1.24	0.49	0.28	11.35	122.50	123.75
	stems	0.80	0.80	0.38	0.16	17.50	195.00	605.00
	roots	1.20	0.92	0.31	0.40	62.50	866.25	2282.50
	whole plant	1.47	1.11	0.43	0.40	24.97	318.90	770.32
Cu hydroxide sulphate	leaves	1.86	0.88	0.57	0.32	11.88	100.00	68.75
	stems	0.86	0.82	0.66	0.10	12.50	105.00	783.75
	roots	1.25	1.03	0.50	0.42	302.50	440.00	1416.25
	whole plant	1.69	0.89	0.56	0.32	56.12	151.31	311.89
Cu hydroxide carbonate	leaves	1.57	0.76	0.30	0.24	9.38	147.50	137.50
	stems	0.87	0.48	0.63	0.10	25.00	125.00	825.00
	roots	1.58	1.06	0.58	0.56	325.00	591.25	2344.38
	whole plant	1.51	0.81	0.36	0.27	57.38	212.01	511.79

Table 4. Biochemical characteristics of lettuce leaves at harvest maturity

Treatment	Free NO_3^-	Vit. C	Carotenoids		Zn	Cu
	mg/1000g FW	mg%	mg/g FW	mg/dm² FW	mg/1000 g FW	mg/1000 g FW
Control	53.75	5.22	0.1986	0.5951	8.72	0.75
Zn hydroxide carbonate	52.50	5.18	0.1851	0.5283	12.48	1.07
Zn hydroxide sulphate	60.55	4.04	0.2292	0.7228	10.29	0.95
Cu hydroxide sulphate	45.00	4.79	0.2062	0.6116	15.60	1.85
Cu hydroxide carbonate	52.17	6.48	0.1894	0.5406	23.45	1.49

Use of the supplement method provides possibilities for a successful comparison of the two sources copper and zinc.

Results from the present investigation lead to the decisive conclusion that hydroxyde sulphates and hydroxide carbonates are equal in value sources of the microelements copper and zinc. In some respects they surpass them: the lower degree of these salts makes them feasible, because the losses are smaller and the pollution of the soil solution and surface waters with free Cu^{2+} and Zn^{2+} ions is decreased.

To the additional advantage of proper endurance and low toxicity is added the stimulating effect of copper and zinc on the growth of the roots.

Conclusions

Hydroxide carbonates and hydroxyde sulphates of copper and zinc, compared to their simple salts, are equal in value as sources of microelements for lettuce. Compared to simple salts hydroxosalts stimulate the growth of the root system.

Hydroxide carbonates are better as a source of zinc, while hydroxyde sulphates as source of copper.

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Reference

Arrambarri, P., O. Garsia, F. Carrera, M. Galzev, 1987. Utilisation de hidroxisales de metales pesados como fuente de micronutrientes para las plantas. Actas del I Simposio National Sobre Nutricion Mineral de la Plantas. Micronutrientes en biologia vegetal y agricultura, 2-3 Abril, 413–421.

Bergmann, W., P. Neubert, 1976. Pflanzendiagnose und Pflanzen analise. VEB Gustav Fisher Verlag, Iena.

Bergman, W., 1988. Ernahrungs storungen bei Kulturpflanzen. VEB Gustav Fisher Verlag, Iena.

Cataldo, D. A., M. Haroon, L. E. Schroder, V. L. Joungs, 1975. Rapid colorimetric determination of nitrate in plant tissues by nitration of salicylic acid. Commun. Soil Science and Plant Analysis, 6(1), 71–80.

Costigan, P. A., 1987. The importance of seedling nutrient stress in producing site to site variations in yield. J. of Plant Nutrition, 10(9–16), 1523–1530.

Ganev, S., 1990. Modern Soil Chemistry. Nauka i iskustvo, Sofia (In Bulg.).

Garbuchev, I., 1978. Regulation of Phosphorus Regime of Basic Bulgarian Soils. Zemizdat, Sofia (In Bulg.).

He Jianqun, Y. Jurong, X. Yialin, C. Yanfeng, 1992. Influence and interactions of the heavy metals on the content of heavy metals in wheat seedlings. J. Ecol., 11, 5–10 (In Chin.)

Ingestad, T., 1970. A definition of optimum nutrient requirements in birch seedlings I. Physiologia Plantarum, 23, 1127–1138.

Ingestat, T., 1971. A definition of optimum nutrient requirements in birch seedlings II. Physiologia Plantarum, 24, 118–125.

Kozhuharov, M., 1960. Colorimetrically determination of phosphorus acid as molybdenum blue with a double reducer stanochloride and hydrazine sulphate. Sciences works of Agricult. Acad. Sci., Agricult. faculty, 8, 27–47 (In Bulg.).

Lyszcz, S., M. Ruszkowska, 1991. Reduction of zinc phytotoxicity in plants with $CaCO_3$. Pam. Pulaw, 99, 179–193 (In Pol.).

Mattingly, G. E. G., 1965. The influence of intensity and capacity factors on the availability of soil phosphorus. In: Soil Phosphorus. Tech. Bull. Minist. Agric. Fish, 13, N.M.S.O. London.

Mirchev, S., 1971. Microelements. Bulg. Acad. Sci., Sofia (In Bulg.)

Stanchev, L., D. Stoyanov, A. Brashnarova, 1982. Microelements and Micro Nutrients. Zemizdat, Sofia (In Bulg.).

Stoyanova, Z., 1988. Uptake, distribution and some peculiarities of zinc metabolism in maize plants. PhD Thesis. Inst. Plant Physiol., Bulg. Acad. Sci. (In Bulg.).

Tyksinski, W., 1992. Response of lettuce plants to difference application of micro nutrient. Rocz. AR 3 pozpr. nauk, 233, 1–67 (In Pol.).