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A POSSIBILITY OF USING HYDROXIDE MIXED CRYSTALS AS SOURCES OF COPPER AND ZINC IN LETTUCE (*LACTUCA SATIVA*) CULTIVATION

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Summary. Copper and zinc are vitally important nutrient elements. A number of Cu and Zn compounds are known applied as microfertilisers and fungicides – sulphates, chelates, lactates, acetates, etc. The aim of the present study was to investigate mixed hydroxide carbonates and hydroxide sulphates of Cu and Zn with different Cu/Zn ratios = 0.35, 1.01, 2.03 (hydroxide sulphates); 0.53, 1.02, 1.98 (hydroxide carbonates), and to chose those most suitable as Cu and Zn source for higher plants. Pot experiments were carried out whit the obtained compounds with the lettuce variety Julta gumurdjinska cultivated as water cultures to phase technical maturity. The results obtained showed that mixed hydroxide sulphates and hydroxide carbonates are good and promising sources of microelements Cu and Zn for plants. From the mixed hydroxide sulphates and hydroxide carbonates studied, best effect showed the hydroxide salts with a ratio in favour of Zn (Cu/Zn = 0.35; 0.53). On the base of these results, a preference could be given to hydroxide carbonates. However the qualities of both compounds make them suitable components of a mixture for presowing treatment of seeds of small-grained agricultural crops.

Key words: mixed hydroxide salts, microelements, Cu, Zn, mineral nutrition

A number of Cu and Zn compounds applied as microfertilisers and fungicides are known – sulphates, chelates, lactates, acetates, etc. All of them possess essential de-

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fects, related to the biological availability of the corresponding ions, based on excessively high or low level of dissociation, inadequate ecological indices, expressed in soil and water pollution with heavy metals and their accumulation in the plant biomass. Copper and zinc are indispensable for higher plants but are potential pollutants, also. One considerable pollution source in the agricultural practice with zinc are the phosphate fertilisers and with copper – plant protection compounds. The limits among deficiency, sufficiency and toxicity for Cu are not very wide. In the tissues it is accepted that up to 4 ppm means deficiency, range 5–20 ppm – sufficiency, and above 20 ppm – toxicity (Fernandes and Henriqes, 1991). Especially sensitive to copper toxicity are the roots of the plants but the reason for that is not clear yet (Hale and Orcuh, 1987). Excess of Cu inhibits the uptake and transport of Fe and besides causes growth reduction chlorosis, too. Among Cu, Zn and Fe exist relations of non-specific absorption competition (Brar and Sekhon, 1976; Swiader, 1985) as for Cu and Zn it is known that the uptake channels are the same (Bowen, 1987). After Fe, Zn is one of the most important metals and second after Ca regulatory element (Clarkson and Zutle, 1989).

The mobility of the nutrient elements in the soil–plant system is regulated not only by their concentration but by their interaction in the nutrient substrate (Jarvis and Whitehead, 1981). Previous studies established that hydroxide chlorides, hidroxide sulphates and hidroxide carbonates can be used as equivalent sources of Cu and Zn of simple salts for higher plants and in some cases are even better (Arrambarri et al.,1987; Stoyanova et al., 1995; Doncheva et al., 1995).

The aim of the present study was to investigate mixed hydroxide carbonates and hydroxide sulphates of Cu and Zn with different ratio Cu/Zn = 0.35, 1.01, 2.03 (hydroxide sulphates); 0.53, 1.02, 1.98 (hydroxide carbonates), and to choose the most suitable for higher plants Cu and Zn source. As a model of plants with small seeds lettuce (*Lactuca sativa*) was used.

The final aim of our research is to apply mixed hydroxide salts as source of Cu and Zn together with other micro- and macroelements as a starter fertilizer for small seed crops.

Materials and Methods

Hydroxide crystals were obtained by the method of partial metal ion (Cu and Zn) precipitation from their mixed sulphate solutions with different ratio of the two ions with NaOH or $NaHCO_3$ at different temperatures (Markov and Joncheva, 1990). Solid phases were precipitated, structurally corresponding to the salts: $Cu_2(OH)_2CO_3$; $Zn_5(CO_3)_2(OH)_6$; $Cu_4(OH)_6SO_4$; $Cu_3(OH)_4SO_4$; $Zn_4(OH)_6SO_4.5H_2O$. The contents of Me^{2+} ions in the obtained compounds were determined by atomic absorption spectrophotometry, complexometrically and iodometrically. XRD analyses were performed by diffractometer using Cu-Ka radiation, monochromatised with banded quartz filter (XR apparatus DROH-1).

The characteristics of the obtained hydroxide sulphates and hydroxide carbonates are given in Table 1.

Table 1. Chemical characteristics of mixed hydroxide salts of copper and zinc

Sample	Cu/Zn	Formula	Dissociation in %	
			3rd day	24th day
1. Control	–	–	–	–
2.	0.35	(I) $Zn_4(OH)_6SO_4$ + (II) $Cu_4(OH)_4SO_4$	26	45
3.	2.03	II + I	14	20
4.	1.01	I + II	18	17
5.	1.02	(III) $(Zn,Cu)_5(OH)_6CO_3$ + (IV) $Zn_5(OH)_6(CO_3)_2$	20	42
6.	1.98	III + IV	8	26
7.	0.53	III + IV	19	40

From the synthesized salts with preliminary given ratio Cu/Zn >1 = 1< 1 was to be established the best one for the growth and development of the plants. Pot experiments were carried out with the obtained compounds using the lettuce cultivar Julta gumurdjinska grown to the phase technical maturity (30 days). The plants were cultivated on nutrient solutions. For this purpose we modified the method of addition of Ingestad (1970, 1971). The mixed hydroxide crystals of Cu and Zn were introduced once in the beginning of the experiment, and their concentration was estimated on the basis of the necessary amount of Zn exceeded to form yield. The ion concentration in the nutrient solution was observed and corrected conductometrically.

The nutrient solution used was especially prepared for lettuce by Georgieva and Nicolova (personal communication) which has the following composition: 2.45 mM $Ca(NO_3)_2 .4H_2O$, 1.33 mM KH_2PO_4, 2.8 mM KNO_3, 3.5 mM $MgSO_4.7H_2O$, 0.17 mM KCl (CNS). Microelements from A to Z according to Hoagland (ME), Fe-EDTA and Mo($Na_2MoO_4.2H_2O$) were added. The studied hydroxide mixed crystals were introduced once at the time of planting the plants according to the pattern:

1. CNS + $CuSO_4$ + $ZnSO_4$ + Fe EDTA + ME (control)
2. CNS + Cu-Zn-hydroxide sulphate Cu/Zn = 0.35 + Fe-EDTA + ME
3. CNS + Cu/Zn-hydroxide sulphate Cu/Zn = 2.03 + Fe-EDTA + ME
4. CNS + Cu/Zn-hydroxide sulphate Cu/Zn = 1.01 + Fe-EDTA + ME
5. CNS + Cu/Zn hydroxide carbonate Cu/Zn = 1.02 + Fe-EDTA + ME
6. CNS + Cu/Zn hydroxide carbonate Cu/Zn = 1.98 + Fe-EDTA + ME
7. CNS + Cu/Zn hydroxide carbonate Cu/Zn = 0.53 + Fe-EDTA + ME

On the third day after placing the plants in the experimental solutions the concentrations of Cu, Zn and Fe in the nutrient solution were measured. The solid phase left was analysed by RD. The hydrogen ions concentration in the nutrient solution was measured on the day of preparation and on the 12th day. The plants were collected in phase technical maturity and were measured biometrically. The data for accumulated fresh biomass and dry matter were processed statistically by the method of analysis of variance. The nitrogen was determined as esmerald green by the apparatus Contiflo after wet digestion with concentrated H_2SO_4 and H_2O_2, and P – as molibden blue colorimetrically by Kojuharov (1960). Ca, Mg, Fe, Cu and Zn were determined after dry ashing and dissolving in 20% HCl by atomic absorption spectrophotometry (Stanchev at al., 1982). The content of free nitrates was determined from dry plant material with 5% salicylic acid according to Cataldo at al. (1975). From the fresh plant material were determined: vitamin C with 2% oxalic acid titrimetrically with dichlorphenolindophenol, and carotenoids – in 80% acetone spectrometrically by Arnon (1949).

Resuls and Discussion

The concentrations of Cu, Zn and Fe in the nutrient solution on the third day of the experiment and at harvesting are a result of the interaction between the components of the nutrient solution, on one hand, and the plant, on the other hand. The Cu and Zn concentrations in the nutrient solution on the third day follow the initial Cu and Zn concentration (Table 2). The dissociation of the hydroxide sulphates on the third day depending on the Cu/Zn ratio was as follows: Cu/Zn = 0.35 – 26%, for Cu/Zn = 1.01 – 18%, and for Cu/Zn = 2.03 – 14%. The hydroxide carbonates dissociation was lower and within the limits: Cu/Zn = 0.53 – 19%, 1.02 – 20% and 1.98 – 8% respectively to the initial concentration (Table 1). The measured Cu and Zn concentrations at har-

Table 2. Characteristics of the nutrient medium (mg/l)

Treatments	Theor. concentration at the beginning		Duration (days)			
			Cu		Zn	
	Cu	Zn	3	24	3	24
Control	0.01	0.02	0.02	0.23	0.46	0.88
Cu/Zn 0.35 xydroxide sulphate	2.48	7.04	0.64	1.14	1.21	2.42
Cu/Zn 2.03 xydroxide sulphate	10.54	5.33	1.52	2.06	2.09	1.32
Cu/Zn 1.01 xydroxide sulphate	6.49	6.59	1.19	1.08	1.52	1.54
Cu/Zn 1.02 xydroxide carbonate	5.61	5.66	1.14	2.32	1.32	2.53
Cu/Zn 1.98 xydroxide carbonate	7.05	3.95	0.59	1.83	0.66	1.87
Cu/Zn 0.53 xydroxide carbonate	3.35	6.40	0.65	1.34	1.61	1.31

Table 3. Biomass accumulated in lettuce at the phase of harvesting, expressed in g/plant

Treatment	Biomass	Leaves	Stems	Roots	Whole plant
Control	FW	32.25 ± 2.68	1.98 ± 0.25	9.37 ± 1.04	43.60 ± 2.27
	DW	3.50 ± 0.29	0.36 ± 0.05	0.66 ± 0.07	4.53 ± 0.28
Cu/Zn = 0.35 hydroxide sulphate	FW	38.33 ± 2.18	2.62 ± 0.56	9.64 ± 0.78	50.58 ± 3.28
	DW	3.14 ± 0.18	0.40 ± 0.08	0.57 ± 0.05	4.10 ± 0.22
Cu/Zn = 2.03 hydroxide sulphate	FW	32.39 ± 1.05	2.18 ± 0.22	9.86 ± 0.88	44.47 ± 2.14
	DW	2.48 ± 0.08	0.26 ± 0.03	0.56 ± 0.05	3.30 ± 0.13
Cu/Zn = 1.01 hydroxide sulphate	FW	32.69 ± 1.77	2.19 ± 0.24	7.30 ± 0.83	42.18 ± 2.27
	DW	3.71 ± 0.20	0.44 ± 0.05	0.31 ± 0.04	4.40 ± 0.21
Cu/Zn = 1.02 hydroxide carbonate	FW	39.21 ± 2.23	2.13 ± 0.22	12.05 ± 0.78	53.39 ± 4.27
	DW	3.22 ± 0.18	0.33 ± 0.04	0.90 ± 0.06	4.54 ± 0.33
Cu/Zn = 1.98 hydroxide carbonate	FW	34.34 ± 1.16	2.25 ± 0.25	10.93 ± 0.41	47.52 ± 2.23
	DW	2.94 ± 0.13	0.42 ± 0.05	0.89 ± 0.03	4.24 ± 0.17
Cu/Zn = 0.53 hydroxide carbonate	FW	38.46 ± 2.49	2.73 ± 0.41	9.48 ± 0.78	50.60 ± 3.58
	DW	5.27 ± 0.34	0.77 ± 0.12	0.86 ± 0.07	6.90 ± 0.47

vesting generally preserved the same tendency, for hydroxide sulphates the values were 45%, 17% and 20% respectively, and for the hydroxide carbonates – 40%, 42% and 26%. In this case the interaction of the ions in the solution was better expressed and part of them precipitated. The nutrient solutions with initial pH 5.6 became alkaline, thus the equilibrium for the different salts was set up within limits 6.64–6.83. The diffraction spectra of the hydroxide salts and the solid phase left after harvesting show that the phase composition does not change, i.e. part of the hydroxide salts do not change their state. This result supplements the data obtained from the analysis that the measured concentrations of the hydroxide salts on the third day and at harvest are lower, compared to these used in the initial stage of the experiment (Table 2).

Data on accumulated fresh biomass and dry matter (Table 3) in our experimental conditions revealed that the three types of salts used, namely simple salts hydroxide carbonates and hydroxide sulphates possess some characteristic properties. A tendency to increasing leaf biomass, which is the valuable part of lettuce, renders hydroxide sulphate with ratio Cu/Zn = 0.35, the hydroxide carbonates with ratio Cu/Zn = 1.01 and 0.53. The differences in the amount of fresh biomass are statistically not reliable, but are near the limits of statistical significance. All hydroxide carbonates have a relative part of the root system increased as comparied to simple salts. In both hydroxide salts groups the compounds with ratio in favour of Zn produce positive effect on the growth of roots and stems.

Data about accumulated dry matter of leaves, stems, roots and whole plants show the same tendency as that of fresh biomass. There are statistically significant differences between the hydroxide sulphates, hydroxide carbonates and the control of the roots. This result suggests that the mixed hydroxide salts of Cu and Zn have a statistically significant effect mainly about the increased growth of the root system.

The N, P, Ca and Mg contents (Table 4) show a tendency to increase in the organs of plants cultivated in the presence of hydroxide sulphates. Similar tendency is maintained by the hydroxide carbonates but is loss expressed.

There is a clearly expressed correspondence between the Cu and Zn concentrations in the nutrient solution, the elements' content in the plant tissues and the degree of dissociation of the salt used (Table 1, 2, 5).

The plants cultivated in nutrient solution containing hydroxide salts with ratio Cu/Zn ≅ 1 have similar distribution of Cu in the organs. Highest Cu content was observed in the roots, followed by stems and lowest – in the leaves. Cu content is highest in the organs of plants cultivated on hydroxide salts with ratio Cu/Zn ≅ 2 (Table 5). According to some authors (Jarvis and Whitehead, 1981; Baszynski et al., 1982; Tukendorf and Baszynski, 1985) in a considerable number of vascular plants roots are the organ which reflects best Cu concentration in the nutrient substrate. Cu contents in the leaves and stems do not show considerable dependence on the change of the elements' concentration in the substrate, our data confirm this statement (Table 2, 5).

Table 4. Chemical composition of lettuce at the phase of harvest maturity

Treatment	Organ	mg/1000g DW			% of DW	
		Fe	Mg	Ca	N	P
Control	leaves	213.12	0.28	0.44	1.86	0.80
	stems	288.75	0.10	0.31	0.87	0.36
	roots	857.50	0.22	0.54	1.10	1.08
	whole plant	313.66	0.23	0.81	2.48	0.81
Cu/Zn 0.35	leaves	110.00	0.31	0.60	2.35	0.94
hydroxide	stems	605.00	0.12	0.24	0.90	0.49
sulphate	roots	1622.50	0.35	0.36	1.43	0.80
	whole plant	368.29	0.29	0.53	2.08	0.87
Cu/Zn 2.03	leaves	178.75	0.36	0.68	2.60	1.05
hydroxide	stems	646.00	0.14	0.32	1.00	0.78
sulphate	roots	2021.25	0.50	0.46	1.65	0.84
	whole plant	406.64	0.37	0.61	2.31	0.99
Cu/Zn 1.01	leaves	151.25	0.32	0.52	2.20	0.82
hydroxide	stems	687.50	0.10	0.30	0.82	0.59
sulphate	roots	2626.25	0.40	0.59	1.65	0.72
	whole plant	377.20	0.30	0.50	2.30	0.79
Cu/Zn 1.02	leaves	199.38	0.29	0.59	1.96	0.84
hydroxide	stems	728.75	0.10	0.48	0.79	0.69
carbonate	roots	1375.00	0.32	0.41	1.07	0.62
	whole plant	466.34	0.28	0.53	1.66	0.81
Cu/Zn 1.98	leaves	247.50	0.29	0.53	1.92	0.89
hydroxide	stems	783.75	0.12	0.55	0.85	0.51
carbonate	roots	955.62	0.29	0.35	1.15	1.08
	whole plant	448.89	0.27	0.49	1.65	0.89
Cu/Zn 0.53	leaves	144.38	0.24	0.43	1.68	0.80
hydroxide	stems	811.25	0.12	0.65	0.84	0.57
carbonate	roots	1588.12	0.43	0.60	1.20	1.02
	whole plants	395.37	0.25	0.47	1.52	0.80

Zn content in the root directly correlates with the Zn concentration in the nutrient solution. Zn contents in the leaves and stems generally follow the concentration in the nutrient solution, but the changes are in considerably narrow limits. Zn is distributed similarly to Cu, with the exception of hydroxide sulphate with ratio Cu/Zn $\cong$ 1.

Plants, cultivated on both hydroxide salts Cu/Zn = 0.35 and Cu/Zn = 0.53 have low Fe content in the leaves and relatively high Fe content in the roots (Table 4). In hydroxide sulphate and hydroxide carbonate with ratio Cu/Zn $\cong$ 1 Fe content in the leaves and roots is higher. Highest Fe content in the leaves is found on the hydroxide

Table 5. Cu and Zn content in lettuce organs at the phase of harvest maturity

Treatment	Cu mg/1000 mg dry weight			Zn mg/1000 mg dry weight		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Control	6.88	12.50	25.62	80.00	200.00	307.00
Cu/Zn 0.35 hydroxide sulphate	14.38	23.75	137.50	200.00	255.00	1210.00
Cu/Zn 2.03 xydroxide sulphate	18.75	30.00	362.50	170.00	310.00	1113.00
Cu/Zn 1.01 xydroxide sulphate	13.75	28.75	362.50	130.00	140.00	1017.00
Cu/Zn 1.02 xydroxide carbonate	20.62	21.25	120.00	130.00	150.00	680.00
Cu/Zn 1.98 xydroxide carbonate	16.25	25.00	147.70	110.00	185.00	859.00
Cu/Zn 0.53 xydroxide carbonate	13.75	16.25	150.00	95.00	110.00	492.00

salts with ratio Cu/Zn = 1.98 and 2.03. The ratio between Cu and Zn shifted in favour to Zn and diminished Fe in the green assimilating parts of the plant. Comparison of the data for accumulated fresh biomass and dry matter with those about Cu, Zn and Fe content in the plant tissues shows that the increased Fe content does not correlate with higher productivity. These results are quite indicative about the interaction of the three elements in the nutrient solution and about Fe activity in the plant.

Data in Table 6 concerning the biochemical characteristics of the leaves show that in all compounds used the free NO_3^- content is very low compared to the permissible NO_3^- content by BSS. Relatively high is the NO_3^- content in the salts with ratio Cu/Zn = 0.35 and 0.53 where the ratio shifts in favour of Zn. At the ratio Cu/Zn ≅ 1 the nitrate content is lower. Highest free NO_3^- content was observed at a ratio Cu/Zn > 1. The hydroxide carbonate source was lower than that in hydroxide sulphate.

Vitamin C and carotenoids content varied within narrow limits and no corelation was observed between the salts used as Cu and Zn sources. The admissible Zn content in the plant products was 0.2 to 18 mg/1000 g fresh weight, and that of Cu – 0.2

Table 6. Biochemical characteristics of lettuce leaves at harvest maturity

Treatment	Free NO^{3-} mg/kg FW	Vit. C mg %	Carotenoids mg/g FW	Carotenoids mg/dm^2 FW	Zn mg/kg FW	Cu mg/kg FW
Control	53.75	5.22	0.1986	0.5951	8.682	0.740
Cu/Zn 0.35 hydroxide sulphate	75.00	4.94	0.1094	0.3435	13.292	1.170
Cu/Zn 2.03 hydroxide sulphate	78.20	5.37	0.1264	0.3897	15.720	1.430
Cu/Zn 1.01 hydroxide sulphate	65.45	4.33	0.1380	0.4368	14.766	1.562
Cu/Zn 1.02 hydroxide carbonate	42.25	3.98	0.1765	0.5333	10.686	1.695
Cu/Zn 1.98 hydroxide carbonate	59.85	4.31	0.2137	0.6125	8.122	1.389
Cu/Zn 0.53 hydroxide carbonate	76.50	5.26	0.2223	0.5992	15.068	1.540

Table 7. Macro- and microelement content in lettuce seeds and plants at transplants phase (mg/plant)

Element	Content in seeds	Content in transplants phase	Ratio content transplants/seed phase (timer)
N	5.92×10^{-3}	2.37	400
P	2.42×10^{-3}	0.97	400
Mg	1.17×10^{-3}	0.38	324
Zn	2.72×10^{-6}	1.40×10^{-2}	500
Cu	6.12×10^{-7}	2.20×10^{-3}	360
Fe	3.13×10^{-6}	1.90×10^{-2}	607

to 30 mg/1000 g fresh weight, according to Bulgarian State Standard (BSS). Zn content in lettuce leaves which are of agricultural interest varies within the limits of 8.122 to 15.72. The obtained production contained copper from 1.176 to 1.692 mg/1000 g or lower then the upper admissible limit, according to BSS. In the mixed hydroxide sulphates and hydroxide carbonates used the obtain production contains copper from 1.176 to 1.692 mg/1000 g, or considerably lower than the sanitary standards.

According to Costigan (1987) the small seed agricultural plants like lettuce grow slowly in the initial period of germination (about 14 days) and later abundant supply with nutrients improved growth. Data in table 7 show that in the period germination – seedling phase Fe content is increased 607 times, Zn – 500, Cu – 360 and N, P – 400. Having in mind this data it can be suggested that Fe and Zn supply proves to be decisive in the first 14 days.

The obtained results show that the mixed hydroxide sulphates and hydroxide carbonates are favourable sources of microelements Cu and Zn for plants. Arrambari et al. (1987) come to the same conclusion about the hydroxide chlorides of Cu and Zn.

Having in mind the hight tolerance of plants, the low toxicity, and lower solubility in comparison to the simple salts and their low price, together with the stimulating effect on the growth of the root system, they could be recommended as alternative to the simple Cu and Zn salts, used until now as microelement sources for higher plants. From the mixed hydroxide sulphates and hydroxide carbonates studied, best effect showed the hydroxide salts, where the ratio is in favour of Zn (Cu/Zn = 0.35; 0.53). This tendency is similar both for hydroxide carbonates and for hydroxide sulphates. Based on all the results presented preference could be given to hydroxide carbonates more than to hydroxide sulphates. Hydroxide carbonates are inferior to the hydroxide sulphates also became they ensure less transported Fe from the root and stem to the leaves. The qualities of both compounds make them suitable component of a mixture for presowing treatment of seeds of small seeded agricultural crops.

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