

Effect of Copper Foliar Spray upon the Contents of Other Elements in Apple Leaves

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Abstract— *Apple leaves of cultivars Topaz and Golden delicious, organically grown upon spindly shaped apple trees and grafted at 5 dwarfing rootstocks, were analyzed for 42 main and trace elements. Spraying a Cu-oxychloride particle suspension plus an adherent as a fungicide, induced some leaf concentration changes with respect to untreated control groups, though inputs of other elements from spraying were negligible. Cu- treatment tended to increase concentrations of Fe, Si and J, and to decrease Zn, Co and Cd in the leaves, because these effects appeared for both cultivars at all rootstocks. Other changes might be rather due to fertilization regime and climate.*

Keywords— *apple leaves, Cu-spraying, trace elements, year-to-year variation.*

I. INTRODUCTION

In order to cope with fungal diseases during organic viticulture and fruit farming, use of foliar copper spraying up to 6 kg/ha.a has been generally permitted within the EU [1]. Fixation of copper-hydroxide or copper-oxychloride particles at leaf surfaces can be achieved by emulsions together with adherents like ethoxylated rapeseed oil or soy oil. Simulation of elution by rain drops showed that adherents decreased Cu-losses from leaves within the first 30 mm of rain got decreased from 75-90% down to 10-25%, and increased the covered surface area per particle. Washout to the soil left the particles rather unchanged [2,3]. Within the soil, the Cu-containing particles get irreversibly adsorbed at humics and pedogenic oxides within a few hours, leaving exchangeable fractions within a few percent of total [4].

Because Cu is much more toxic towards fungi than towards bacteria, Cu inputs decreased fungal biomass, whereas bacterial phospholipids and xylanase were not affected, but effects upon the mycorrhiza remained unclear. Thus, effects upon bacterially driven N-, P- and C- cycles seem less pronounced [5].

Though numerous studies about Cu-speciation and mobilities in soil, as well as toxicity symptoms in green plants are available, effects upon the metabolism of other elements after Cu-spraying are largely unknown.

In green plants, about 98% of total Cu is bound to various organic molecules. Cu shows high affinities to thiol groups of peptides, and thus to proteins rich in cystein. But it may also form stable chelates with carboxylic and hydroxylic groups, often assisted by groups containing basic nitrogen. Cu-containing enzymes catalyse electron transfer reactions, like photosynthesis, respiration, perception of ethylene, metabolism of reactive oxygen, and remodeling of cell walls. Many Cu-proteins have a functional counterpart that uses Fe [6,7].

Whereas divalent Cu shows high affinity to histidine, monovalent Cu favors cystein or methionine. In case, metals are transported by a common transport protein, Cu can displace other essential metals in metallo-proteins because of its high stability of its thiol complexes. According to the Irving-Williams-series, the stability of bondings between metallo-proteins and metals increases within $Ca^{2+} < Mg^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} - Cu^{2+} - Cu^+$ [7,8].

Small organic molecules like mugeinic acid or nicotianamine (N-N-(3-amino-3-carboxypropyl)-3-amino-3-carboxypropyl) azetidine-2-carboxylic acid) are utilized to transport essential metals like Cu, Fe, Mn, Ni or Zn within the green plant. In vitro, the complexation capability of nicotianamine increases within the sequence Mn-Fe-Co-Zn-Ni-Cu, and peaks at pH 6,5. In case of Cu, Fe or Zn deficiencies, nicotianamide get increasingly formed, but this necessitates, however, high supply of nitrogen [6]. At Cu-deficiency, Cu-treatment induces the formation of metallothioneins, which assist the reconstruction of plasma membranes and act as anti-oxidants [9]. Within the roots, both Cu and Fe get reduced by root cell ferric reductase. When given in excess, plants have reduced Fe uptake, and vice versa [6]. Excess Cu concentrations tend to decrease root growth because of preferential Cu- accumulation in that organ.

In case Cu is taken from the soil, the most frequent symptom of Cu- intoxications is chlorosis as well as reduced uptake of Fe. In addition to chlorosis, excess Cu causes symptoms like necrosis, and reduced growth. Inside the cells, excess Cu can disrupt protein structures, reduce enzyme activities, interfere in the biosynthesis of photosynthetic pigments and membranes, cause deficiencies of other essential elements, and induce oxidative effects [8]. Cu-induced Fe deficiency, replacement of Mg by Cu, or destruction of the oxygen transporting polypeptide decreases chlorophyll content.

Therefore, the Cu- tissue levels are regulated within a narrow physiological range by homeostasis. Frequently, green plants have specific metal sensors, which start a cascade of signals to induce corresponding reactions. The green plant can protect itself against excess Cu by stimulation of excretion, increase of chelators, and separation into a vacuole [8].

Immissions of toxic amounts of Cu can be caused by industrial and residential wastes, pig manure and poultry dung, and also Cu foliar sprays. Most papers about metal tolerance deal with elevated soil or hydroponic Cu levels. Among green plants, tolerance versus excess Cu is highly variable. Cu tolerant plants are mainly excluders, reacting by reduced secretion of root exudates, and immobilisation inside the root. Excess soil Cu gets at first enriched within the roots, lowers root growth, promotes root damage and lowers transport processes inside the plant. Transport of excess Cu from roots to shoots gets prevented by adherence to cell walls, reduced flux across plasma membranes, increased outflow from the cytoplasm as well as intracellular chelation by organic acids, special phytochelatin, and metallo-thioneins [7].

The reverse transport of Cu from the leaves back to stalks and roots takes place at leaf aging. Increased N-supply delays aging and affects availability and mobility inside the plant by binding more Cu to amino acids and proteins. Cu gets hardly redistributed from old leaves to younger ones [6,7].

In case of Cu-deficiency, because of limited mobility from soil, foliar Cu-spray acts much faster and more effectively than additions to the soil. Cu levels applied as fungicides, however, are 10-100 times higher than usually needed for fertilization in case of deficiencies [7]. A hydrophobic cuticula protects leaves from external damage. Because the cuticula of young leaves is not so strong, effects of foliar spraying are higher in this case. The adherence of sprayed solutions depends on genotypical differences of leaf surface properties, like hairs and smoothness of surface.

A known interelement effect facilitates the decision to spray each element separately, or to use a mixture and thus safe work. The uptake of foliar-sprayed Cu, Mn, and B into apple leaves was higher in May than in June and September, and elevated levels of Cu lasted longer than of Mn and B. In combination with Mn, the leaves adsorbed less Cu than without [10]. Cu addition to sandy soil also increased the uptake of Ba, Ca, Sr and Fe into the leaves of MacIntosh apple seedlings, but decreased Mn and Mo. Addition of peat to this sandy soil increased soil adsorption, but also decreased Cu uptake into the seedlings [11].

In order to document differences in root trace element uptake and foliar spray, young nursery-grown apple trees were grown in pot experiment on quartz sand. Spraying increased the Cu content of the components that were directly exposed, but hardly increased the Cu content of other tree components, like roots. Differences in plant growth were marginal. The levels of N, P, K, Ca, Mg and Na in the leaves were about the same after sufficient spraying or soil additions of Cu, Mn, Zn and B [12].

Within a field trial at the experimental orchard Jedlersdorf (Vienna/Austria), run by the University of Natural Resources Vienna, apples of „Topaz“ cultivar have been grafted upon different rootstocks, beneath a lot of other fruit items. Rootstocks M9 with and without „Rubinola“ as interstem, M26, M7 grafted at 25 cm and at 55 cm, MM111 and Bittenfelder seedlings, were used, trained as spindles. Growth, yields and mean fruit weights have been reported elsewhere [13]. This enabled us to investigate the effect of rootstocks and the year of growth upon the composition of fruits and leaves at the same site and fertilization regime [14]. In 2012, young leaves were sampled in early June, which had got no Cu-treatment, whereas in 2015 and 2016, young leaves from the same trees were sampled after Cu treatment. At the same site, apple leaves of Golden delicious variety were available with and without Cu treatment, grown in 2016.

For Cu treatment, Cuprofor liquid, containing copper oxichloride, was used, diluted at 0,03% solution (%v/v).

II. MATERIAL AND METHODS

At the experimental orchard Jedlersdorf (Vienna/Austria) in autumn 2008, 5 rows of spindly shaped apple trees were grafted by variety Topaz at dwarfing rootstocks M9, M26, M7 and M111, as well as on seedling (Bittenfelder). At present, Topaz is the most utilized apple cultivar grown by organic farming in Austria. It has favorable storage properties, high vitamin C

contents and a balanced acid-sugar proportion, early flowering stage and late harvest [15]. The trees have been organically grown within 5 rows, each containing 4 trees of each kind randomly distributed.

The soil is of calcareous chernozem type, pH 7.6 -7.8, containing about 7% of total Ca, and low mobile K (in 0,16M acetic acid 1 to 20: 137 mg/kg in the upper 25 cm, and 42 mg/kg below). Fertilization was done by addition of the organic fertilizer "Biofert" (Austria) to supply N at 30 kg/ha.a .

At the day of sampling, leaves of each kind were taken separately within each row, wearing gloves, preferably the third leaf off the sprout tops. All 4 trees within one row were sampled all around, to average light and shadow sites. This enabled to compare the uncertainties between data obtained from different rows and rootstocks, respectively. In the evening after sampling, the leaves were rinsed with de-ionized water upon nylon gauze, put into new poly-ethylene bags, submitted to freeze drying, and finely crashed inside the bags in order to avoid further contacts.

All samples were submitted to two different digestion procedures, at least in duplicate. About 0,25g sample was digested with 3,8 ml suprapure HNO₃ plus 0,1 ml HF p.a. in closed vessels by microwave heating, and finally made up to 25 ml. In addition, about 1 g sample was digested with 8 ml of a nitric acid – potassium chlorate solution (20g KClO₃ p.a. + 200 ml H₂O + 80 ml HNO₃ suprapure), and finally made up to 25 ml also [16]. Ultrapure water and polythene volumetric flasks were used throughout.

The resultant digestion solutions were submitted to multi-element analysis by ICP-OES (Perkin-Elmer Optima 3000XL) operating with a horizontally mounted torch, as well as ICP-MS (Perkin Elmer Sciex ELAN DRC II) for selected low level trace elements. KClO₃ digestion solutions were analyzed at the ICP-OES versus matrix matched calibrants, others with calibrants containing K, Ca and P within expectable ranges. For ICP-MS measurements, samples were diluted 1+9, indium added as internal standard, and read for the elements Bi, Cd, Co, Mo, Ni, Pb, Tl, Y and Rare Earth elements). Total iodine was determined in special runs at higher plasma power than the default, after dilution with 1/80 diluted digestion reagent solution, and standard addition of iodate calibrants.

The KClO₃ digest is especially useful for the determination of non-metals B, Si, S and I, because they get partially volatilized from conc. HNO₃ [16]. Unexpected purity of the KClO₃ permitted the determination of all main and trace elements, except K, Rb and Cs.

The fungicide Cuprofor was refluxed with HNO₃ or HCl, made up to 100 ml, and measured at the ICP after various dilutions and for Cu by flame-AAS. An oily precipitate was filtered off. HNO₃ and HCl yielded the same results.

Total nitrogen contents of the leaves were obtained by combustion utilizing a LECO FP-528 Nitrogen-Analyzer.

III. RESULTS AND DISCUSSION

The Cu-containing fungicide was quite pure with respect to other inorganics, and thus contributed negligible loads of other elements to the leaves. It contained less than 5% of Mg, Ca, Sr and Mn concentrations encountered in dry leaves, as well as Li, Al, Fe, Pb and Ni in slight excess (Table 1). This could not increase leaf concentrations substantially.

TABLE 1
CONCENTRATIONS FOUND IN CUPROFOR PER WEIGHT

Cu 28.9 ± 1.7 %			
	mg/kg		mg/kg
Na	1535 ± 187	Cr	8.5 ± 1.1
Ca	613 ± 43	Cd	2.0 ± 0.5
Fe	287 ± 35	Pb	1.7 ± 0.5
Zn	198 ± 20	Co	1.7 ± 0.5
K	158 ± 103	Mn	1.1 ± 0.4
Al	154 ± 54	Ni	1.1 ± 0.4
Mg	47 ± 16	Sr	0.85 ± 0.15
		Li	0.32 ± 0.10

Whereas in leaves taken in 2012, Cu levels were ambient, Cu in leaves taken 2015 ranged about 100-150 mg/kg, which would be highly toxic, if the same load had come via the soil, because of irreversible root damage. It exceeds the legal limit of 90 mg/kg, given in the Austrian standard ÖNORM S 2203, entitled "Requirements for manufactured soils from compost" [17]. Leaves sampled in 2016 contained about 50-70 mg/kg Cu.

Trends of element concentrations induced by Cu-spraying seem to be real, if the same effects occur upon all rootstocks, as well as within several growing seasons and cultivars.

When concentration ranges met in apple leaves and grown upon various rootstocks, are depicted as boxplots, possible differences get easily visualized, but contrary to Table 2, medians and the within 50%-ranges are shown. Figs. 1-7 show some boxplots assorted to rootstocks, year and cultivars, for those elements, which yielded equal patterns in subsequent years. These patterns were shifted in parallel with respect to 2012 data to higher values in 2015 and 2016 in case of Fe, Si and I, and to lower values in case of Cd, Co, Zn, and possibly Mo and Ti (table 2; figs 1-7). The Rare Earth elements are generally too low to detect such effects. Adverse trends in the two cultivars appeared for N, P as well as for Na, Sr, Ni, Pb, and V, if data from leaves grown upon rootstocks M9 are compared (Table 2); they are surely no effect of Cu-spray but of the fertilization regime. Other elements did not show uniform trends and thus do not seem to be affected by Cu spraying. Also, element proportions like Ca/Mg, Ca/Mn, and B/Mo did not show common trends.

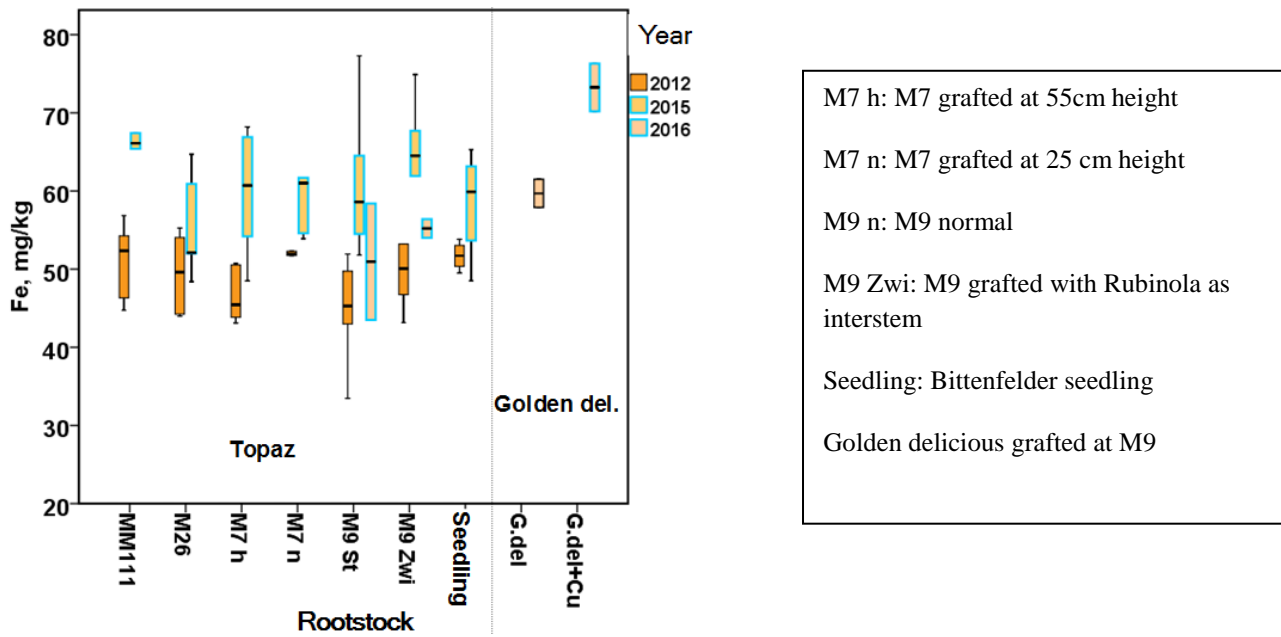


FIG 1. Fe CONTENTS IN APPLE LEAVES!

blue framed boxes: with Cu; black framed boxes: without Cu

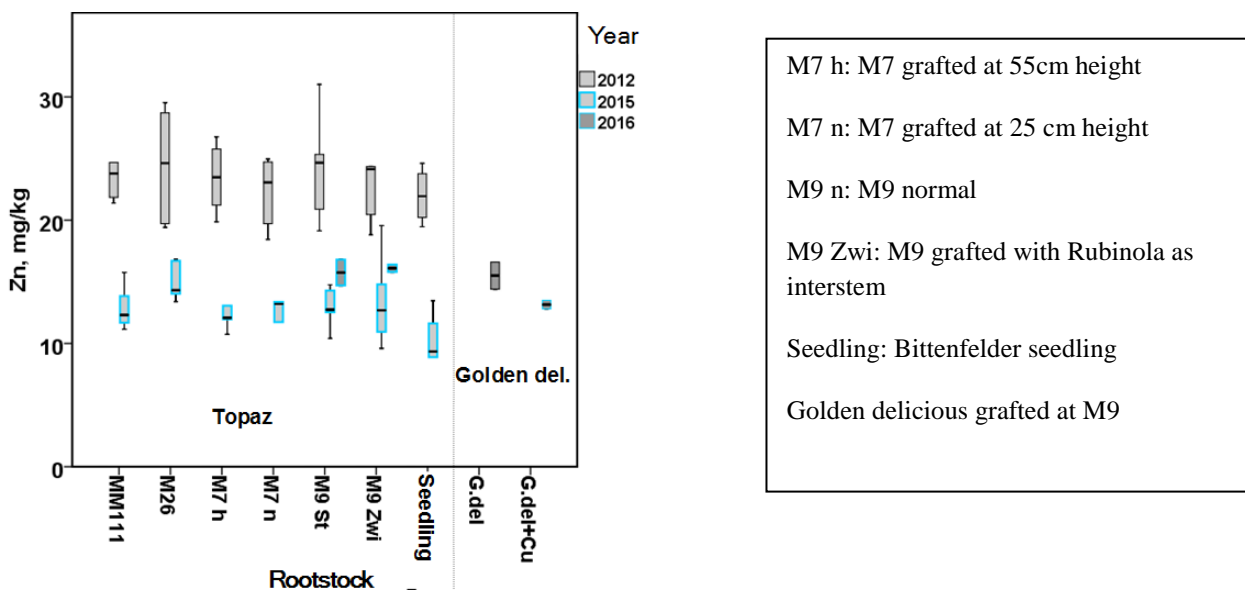


FIG. 2 Zn CONTENTS IN APPLE LEAVES!

blue framed boxes: with Cu; black framed boxes: without Cu

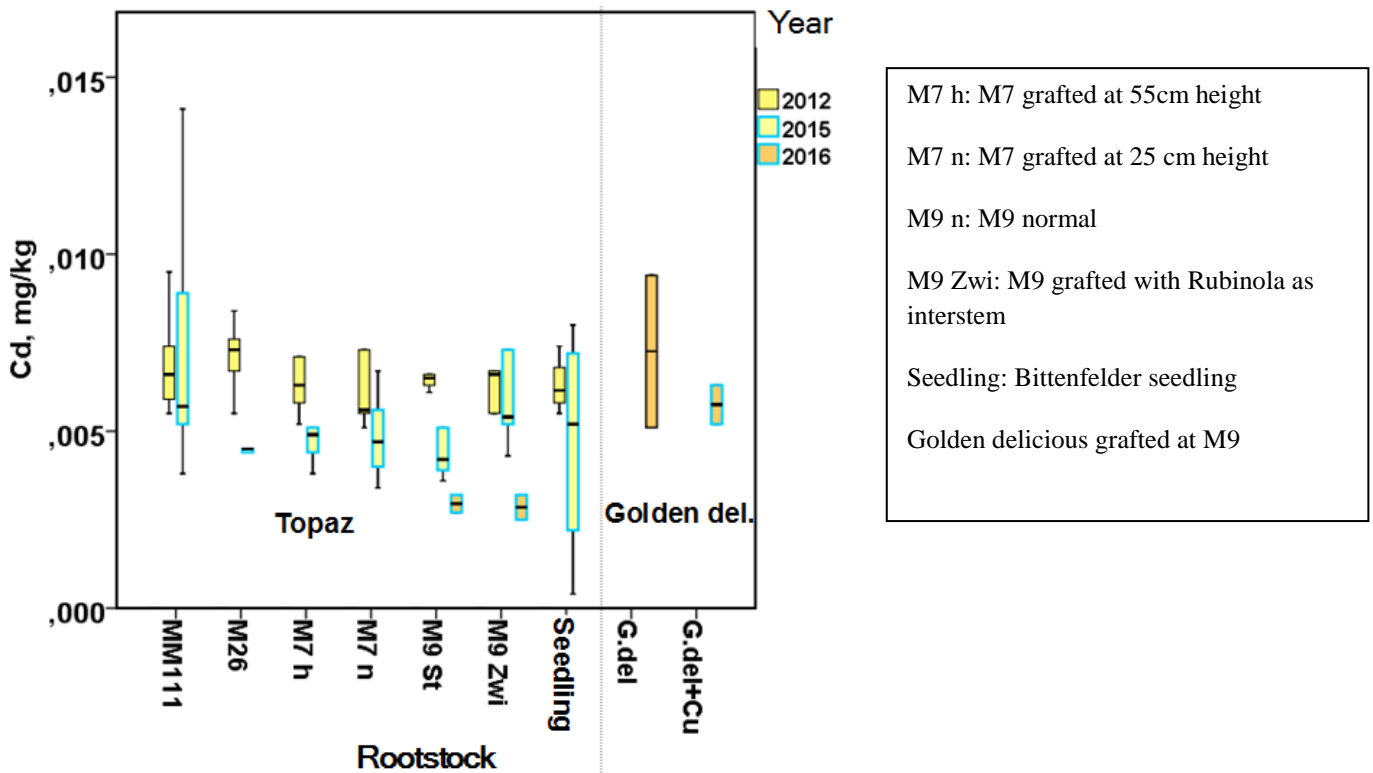


FIG 3. Cd CONTENTS IN APPLE LEAVES!

blue framed boxes: with Cu; black framed boxes: without Cu

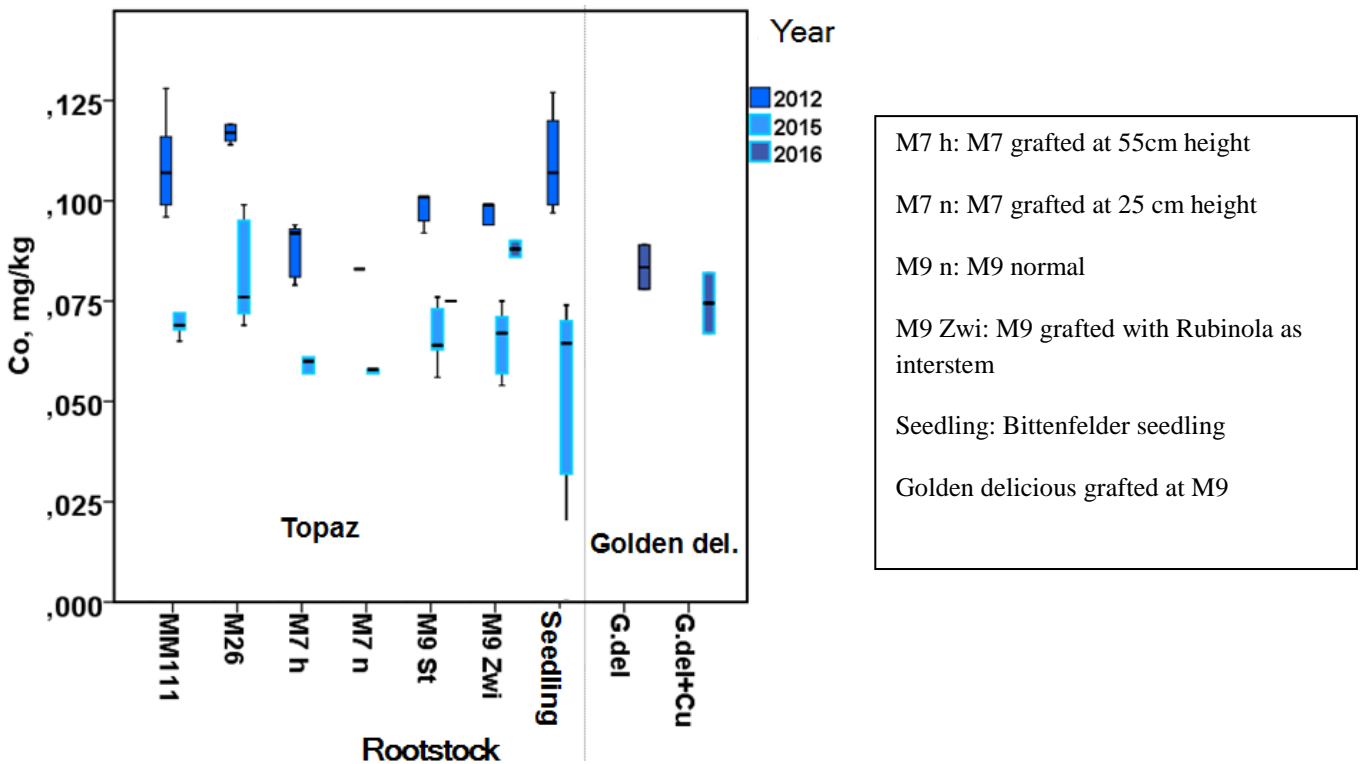
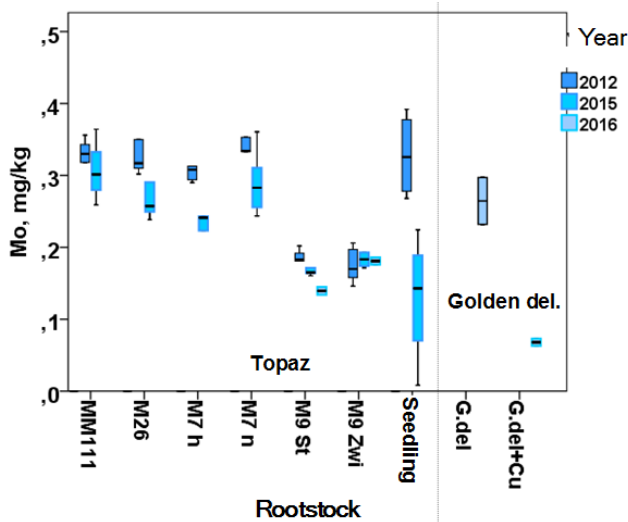


FIG 4. Co CONTENTS IN APPLE LEAVES

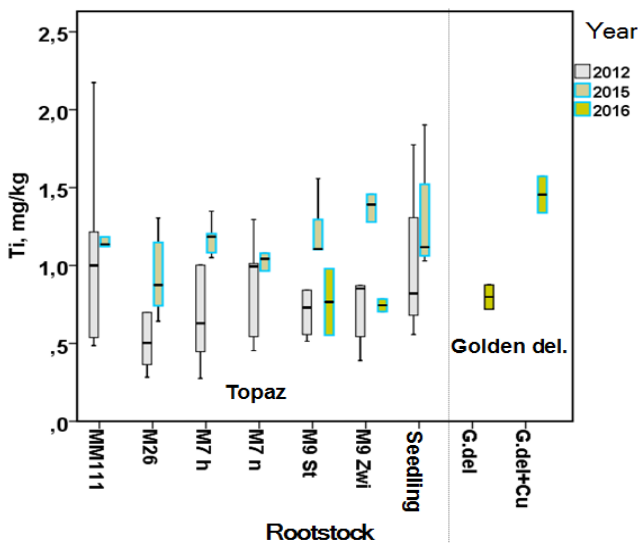
blue framed boxes: with Cu; black framed boxes: without Cu



M7 h: M7 grafted at 55cm height
 M7 n: M7 grafted at 25 cm height
 M9 n: M9 normal
 M9 Zwi: M9 grafted with Rubinola as interstem
 Seedling: Bittenfelder seedling
 Golden delicious grafted at M9

FIG 5. Mo CONTENTS IN APPLE LEAVES

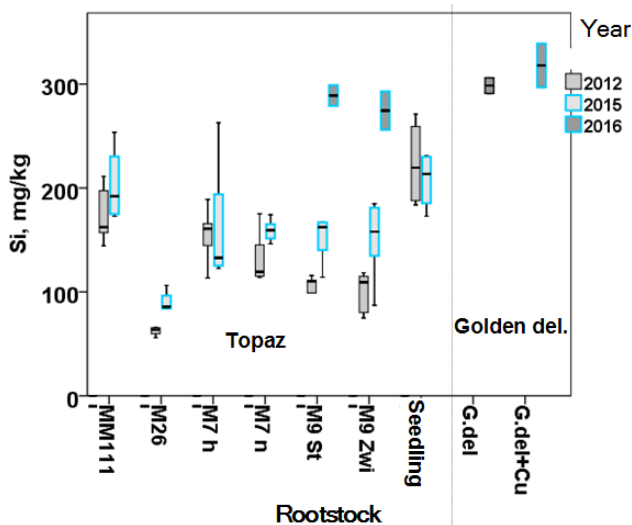
blue framed boxes: with Cu; black framed boxes: without Cu



M7 h: M7 grafted at 55cm height
 M7 n: M7 grafted at 25 cm height
 M9 n: M9 normal
 M9 Zwi: M9 grafted with Rubinola as interstem
 Seedling: Bittenfelder seedling
 Golden delicious grafted at M9

FIG 6. Ti CONTENTS IN APPLE LEAVES

blue framed boxes: with Cu; black framed boxes: without Cu



M7 h: M7 grafted at 55cm height
 M7 n: M7 grafted at 25 cm height
 M9 n: M9 normal
 M9 Zwi: M9 grafted with Rubinola as interstem
 Seedling: Bittenfelder seedling
 Golden delicious grafted at M9

FIG 7. Si CONTENTS IN APPLE LEAVES

blue framed boxes: with Cu; black framed boxes: without Cu

TABLE 2
CONCENTRATIONS IN APPLE LEAVES, mg/kg DRY MASS, GRAFTED AT ROOTSTOCK M9 ASSORTED FOR MAIN ELEMENTS, Cu, ESSENTIALS, AND NON-ESSENTIALS

	Topaz 2012	Topaz 2015	Topaz 2016	Golden del. 2016	Golden del. 2016
% N	2.82 ±0.15	2.65 ±0.22	2.19 ±0.04	2.37 ±0.04	2.50 ±0.04
% Ca	1.313 ±0.135	1.331 ±0.227	1.974 ±0.184	1.673 ±0.155	1.529 ±0.035
% K	0.707 ±0.082	0.684 ±0.140	1.122 ±0.127	1.909 ±0.542	1.925 ±0.075
% Mg	0.388 ±0.035	0.358 ±0.043	0.391 ±0.038	0.266 ±0.016	0.299 ±0.004
% S	0.230 ±0.037	0.193 ±0.008	0.139 ±0.012	0.148 ±0.003	0.149 ±0.010
% P	0.200 ±0.015	0.192 ±0.013	0.162 ±0.024	0.139 ±0.004	0.148 ±0.001
mg/kg					
Cu	10.42 ± 1.16	131.0 ±39.2	50.6 ±4.5	8.83 ±0.35	71.0 ±1.1
Fe	48.3 ±8.5	62.5 ±9.3	53.1 ±6.6	59.7 ±2.5	73.3 ±4.3
Mn	37.8 ±4.1	40.1 ±6.7	27.5 ±2.0	42.9 ±0.3	35.7 ±0.4
Zn	24.1 ±4.6	13.2 ±2.9	15.9 ±0.9	15.5 ±1.6	13.2 ±0.5
B	27.9 ±2.0	23.4 ±3.5	26.7 ±0.8	30.8 ±0.1	30.2 ±0.9
Mo	0.180 ±0.020	0.189 ±0.039	0.160 ±0.025	0.265 ±0.046	0.068 ±0.007
Co	0.099 ±0.009	0.066 ±0.008	0.082 ±0.008	0.084 ±0.008	0.075 ±0.011
Li	0.326 ±0.064	0.308 ±0.049	0.641 ±0.029	1.573 ±0.170	0.665 ±0.025
Na	35.1 ±13.4	22.8 ±11.8	9.8 ±0.7	11.8 ±4.2	15.4 ±0.3
Rb	3.48 ±0.45	3.95 ±2.46	3.80 ±1.06	0.88 ±0.29	4.00 ±0.49
Cs	0.018 ±0.003	0.023 ±0.007	0.020 ±0.001	0.005 ±0.001	0.013 ±0.003
Be	< 0.003	< 0.003	< 0.003	< 0.003	0.004 ±0.001
Sr	26.0 ±4.7	28.0 ±5.4	32.3 ±2.8	42.6 ±13.8	24.6 ±0.1
Ba	44.5 ±7.9	37.9 ±7.0	52.8 ±2.5	25.7 ±1.6	28.6 ±0.7
Al	32.8 ±4.6	32.7 ±8.3	68.9 ±5.5	61.3 ±6.7	84.1 ±5.6
Sc	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Y	0.014 ±0.002	0.017 ±0.004	0.015 ±0.001	0.015 ±0.004	0.029 ±0.014
La	0.027 ±0.011	0.029 ±0.007	0.023 ±0.003	0.031 ±0.003	0.058 ±0.015
Ce	0.051 ±0.008	0.057 ±0.016	0.044 ±0.005	0.061 ±0.000	0.121 ±0.023
Pr	0.006 ±0.002	0.007 ±0.002	0.005 ±0.001	0.006 ±0.001	0.013 ±0.003
Nd	0.021 ±0.009	0.026 ±0.007	0.019 ±0.002	0.025 ±0.002	0.050 ±0.014
Sm	0.004 ±0.002	0.005 ±0.001	0.004 ±0.001	0.004 ±0.001	0.009 ±0.003
Eu	0.010 ±0.001	0.007 ±0.002	0.011 ±0.001	0.007 ±0.003	0.007 ±0.001
Gd	0.004 ±0.001	0.004 ±0.001	0.004 ±0.001	0.004 ±0.0001	0.008 ±0.003
Tb	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Ho	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Er	0.0015±0.0003	0.0018±0.0004	0.0013±0.0005	0.0010 ±0.0000	0.0070 ±0.0071
Lu	< 0.0005	< 0.0005	< 0.0005	< 0.0005	< 0.0005
Ti	0.89 ±0.54	1.27 ±0.48	0.76 ±0.18	0.80 ±0.11	1.46 ±0.16
V	0.052 ±0.014	0.031 ±0.028	0.031 ±0.008	0.037 ±0.006	0.078 ±0.025
Cr	0.065 ±0.041	0.174 ±0.082	0.056 ±0.014	0.058 ±0.022	0.099 ±0.003
Ni	0.29 ±0.07	1.63 ±0.27	0.69 ±0.09	2.12 ±0.18	0.92 ±0.01
Cd	0.0064±0.0014	0.052 ±0.0016	0.0029±0.0004	0.0073 ±0.0030	0.0058 ±0.0008
Si	101 ±18	156 ±40	282 ±19	299 ±11	318 ±30
Pb	0.18 ±0.02	0.24 ±0.03	0.26 ±0.02	0.24 ±0.05	0.22 ±0.06
J	0.263 ±0.087	0.487 ±0.094	0.421 ±0.057	0.317 ±0.001	0.400 ±0.022

IV. CONCLUSION

Cu- spraying as a fungicide increased Cu contents in apple leaves to levels, which would be toxic, if this load would have passed through the roots. Though the input of other elements to the leaves was negligible, Cu-spraying might influence the contents of other trace elements in the leaves, like Fe, Si, I, Cd, Co and Zn. This pilot study has to be confirmed by sampling of treated and untreated leaves for the same cultivars at the same sites within the same years. Future results would be interesting for other fruits and vine as well.

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