Environmental Science and Pollution Research
https://doi.org/10.1007/s11356-020-09151-y

RESEARCH ARTICLE

Field evaluation of one Cu-resistant somaclonal variant and two clones of tobacco for copper phytoextraction at a wood preservation site

Aliaksandr Kolbas[1,2] · **Rolf Herzig**[3] · **Lilian Marchand**[1] · **Jean-Paul Maalouf**[1] · **Natallia Kolbas**[2] · **Michel Mench**[1]

Received: 28 January 2020 / Accepted: 4 May 2020

Abstract

A Cu-resistant somaclonal tobacco variant (NBCu 10-8-F1, C1), its BaG mother clone (C3), and the FoP tobacco clone (C2) were cultivated at a wood preservation site on Cu-contaminated soils (239–1290 mg Cu kg^{-1} soil range) and an uncontaminated control site (CTRL, 21 mg Cu kg^{-1}) to assess their shoot DW yields and potential use for bioavailable Cu stripping. The Cu concentration in the soil pore water varied between 0.15 and 0.84 mg L^{-1}. Influences of Cu exposure and soil treatments, i.e., untreated soil (Unt), soils amended with compost and either dolomitic limestone (OMDL) or zerovalent iron grit (OMZ), on plant growth and shoot ionome were determined. All transplants survived and grew even at high total soil Cu. Shoots were harvested after 3 months (cut 1). Subsequently, bottom suckers developed and were harvested after 2 months (cut 2). Total shoot DW yield (cuts 1 + 2) varied between 0.8 and 9.9 t DW ha^{-1} year^{-1} depending on tobacco cultivars, soil treatments, and soil Cu exposure. It peaked for all cultivars in the OMDL plots at moderate Cu exposure (239–518 mg kg^{-1} soil), notably for the C2 plants. Cut 2 contributed for 11–43% to total shoot DW yield. Increase in shoot DW yield diluted shoot Cu concentration. At low Cu exposure, total shoot Cu removal peaked for the variant. At moderate Cu exposure, shoot Cu concentrations were similar in all cultivars, but total shoot Cu removal was highest for the C2 plants. At high Cu exposure (753–1140 mg kg^{-1}), shoot Cu concentrations peaked for the C2 plants in the Unt plots, the C1 and C2 plants in the OMZ plot, and the C3 ones in the OMDL plots. Shoot Cu removal (in g Cu ha^{-1} year^{-1}) ranged from 15.4 (C2 on the CTRL soil) to 261.3 (C2 on moderately contaminated OMDL soils). The C2 plants phytoextracted more Cu than the C1 and C3 ones in the Unt plots and in the OMDL plots at moderate Cu exposure. In the OMDL plots with high Cu exposure, shoot Cu removal was highest for the C1 plants. Soil amendments improved shoot Cu removal through increase in either shoot DW yield (OMDL—3-fold) or shoot Cu concentration (OMZ—1.3-fold). Increased shoot Cu concentration induced an ionome imbalance with increased shoot Al, Fe, B, and Mg concentrations and decreased P and K ones. Copper concentrations in plant parts varied in decreasing order: roots > leaves > inflorescence (cymes including seeds) > stem, whereas Cu removal ranked as roots > stem = leaves > inflorescence.

Keywords Compost · Bioavailable metal stripping · Iron oxides · Liming · *Nicotiana tabacum* L. · Phytomanagement · Phytoremediation · Soil amendment

Responsible editor: Elena Maestri

✉ Michel Mench
michel.mench@inrae.fr

Aliaksandr Kolbas
kolbas77@mail.ru

Rolf Herzig
rolf.herzig.agb@bluewin.ch

[1] Univ. Bordeaux, INRAE, BIOGECO, 33615 Pessac, France

[2] Brest State University named after A.S. Pushkin, Boulevard of Cosmonauts 21, 224016 Brest, Belarus

[3] Phytotech Foundation, Quartiergasse, 12, 3013 Bern, Switzerland

Abbreviations

APX	Ascorbate peroxidase
C1	Cu-resistant somaclonal tobacco variant (NBCu 10-8-F1)
C2	Tobacco clone Forchheimer Pereg (FoP)
C3	Tobacco mother clone Badischer Geudertheimer (BaG)
CCA	Chromated copper arsenate
CEC	Cation exchange capacity
CPM	Compost made of poultry manure and pine bark chips
CTRL	Control soil

CuR	Shoot Cu removal
CuSH	Shoot Cu concentration
CuSPW	Total Cu concentration in the soil pore water
CuT	Total soil Cu
DHAR	Dehydroascorbate reductase
DL	Dolomitic limestone
DOM	Dissolved organic matter
DW	Dry weight
EC	Effective concentration
EU	European Union
ISE	Ion selective electrode
OM	Organic matter
OMDL	Compost + dolomitic limestone
OMZ	Compost + zerovalent iron grit
PAH	Polycyclic aromatic hydrocarbons
NA	Nicotianamine
PCA	Principal component analysis
ROS	Reactive oxygen species
SDWY	Shoot dry weight yield
SFWY	Shoot fresh weight yield
SL	Shoot length
SOD	Superoxide dismutase
TE	Trace elements
TOC	Total organic carbon
UNT	Untreated soil
WC	Water content
WHC	Water holding capacity
Z	Zerovalent iron grit

Introduction

The use of Cu-based salts as wood preservatives has resulted in Cu-contaminated soils at many wood preservation sites (Bes et al. 2010; Frick et al. 2019; Tardif et al. 2019). Parts of the EU affected by Cu accumulation in soils due to other human activities (e.g., mining and smelting, fungicides, manufacturing processes, etc.) and factors influencing Cu distribution in topsoils are also reported (Villanneau et al. 2008; El Hadri et al. 2012; Tóth et al. 2016; Ballabio et al. 2018). Although Cu is an essential cofactor in many physiological processes of plants, e.g., photosynthesis, mitochondrial respiration, oxidative stress responses, and transduction of ethylene signal, excessive Cu exposures impact the plant growth (Yruela 2009; Printz et al. 2016; Shahbaz and Pilon 2019). Sparse plant cover and low plant diversity commonly occurred on highly Cu-contaminated soils (Ginocchio et al. 2004; Bes et al. 2010; Lillo-Robles et al. 2019). Soil erosion from barren area, Cu leaching from contaminated topsoils, and transfer into food chains can endanger animal and human health and can impact ecosystem services (Maderova et al. 2011; Marchand et al. 2011; Antoniadis et al. 2019; Rehman et al. 2019; Taylor et al. 2020).

There are various phytomanagement options for reducing the pollutant linkages associated to Cu-contaminated soils. In situ stabilization and aided phytostabilization have been evaluated in field trials to sustainably minimize the dispersion and biological action of bioavailable soil Cu in excess and to promote a vegetation cover on Cu-contaminated soils, notably at wood preservation sites (Cordova et al. 2011; Kumpiene et al. 2011, 2019; Carcamo et al. 2012; Lagomarsino et al. 2011; Pardo et al. 2018; Xue et al. 2018). Phytoextraction, i.e., bioavailable contaminant stripping by harvested plant parts, combined with incorporation of soil conditioners (so-called aided phytoextraction) is another option (Kolbas et al. 2011; Mench et al. 2018; Burges et al. 2020). Merging together the cultivation of non-food crops for supplying plant-based feedstock, the amelioration of metal(loid)-contaminated soils, and risk management is developing at field scale (Vangronsveld et al. 2009; Meers et al. 2010; Mench et al. 2010a, 2018; Witters et al. 2012; Herzig et al. 2014; Kidd et al. 2015; Cundy et al. 2016; Thijs et al. 2018).

Up to our knowledge, no Cu hyperaccumulators are validated in field trials for Europe, although the Cu-tolerant Diyarbakır ecotype of *Brassica nigra* L. (Cevher-Keskin et al. 2019) and *Hirschfeldia incana* (L.) Lagr.-Foss. ecotypes (Mench et al. 2017) are under investigations. Even though *Elsholtzia splendens* Nakai ex F. Maek. from China is claimed as a Cu-hyperaccumulator (Li et al. 2018), a Chinese population cultivated in potted Cu-contaminated soils from a French wood preservation site (3.5–2600 mg Cu kg^{-1}) displayed small plants after a 2-month growth period, with no visible foliar symptom but low shoot biomass (0.51 ± 0.09 g DW plant^{-1}) and 72.4 mg Cu kg^{-1} in shoots and 2675 mg Cu kg^{-1} in roots, being not relevant for Cu phytoextraction at field scale (Mench et al. 2010b). Bioaugmentation with plant growth–promoting rhizobacteria and/or bacterial endophytes, sometimes combined with chelating agents, are other options assessed for Cu-phytoextraction (Benizri and Kidd 2018; Abbaszadeh-Dahaji et al. 2019; Ren et al. 2019). Chemically induced (hyper)accumulation is however impaired by various environmental risks, e.g., metal leaching from the root zone and toxic effects on microbes (Nowack et al. 2006; Mench et al. 2010a). Regarding secondary Cu-accumulators, few long-term field evaluations of suitable (non-food) crops adapted to local conditions and of their agronomic management are available (Faessler et al. 2010a; Mench et al. 2018; Burges et al. 2020).

Commercial cultivars and mutant lines of sunflower were assessed at a wood preservation site after incorporation of compost and dolomitic limestone into Cu-contaminated soils: shoot DW yield (SDWY—0.2–7, 0.4–5.3 t ha^{-1} year^{-1}), shoot Cu removal (i.e., 3–59, 19–88 g Cu ha^{-1} year^{-1}), and oilseed yield (0.02–3.9 t ha^{-1} $year^{-1}$) depended on sunflower types, Cu exposure, and annual climatic conditions (Kolbas et al. 2011; Mench et al. 2018). Sunflower cropping, however, must be interrupted by crop rotation to avoid allelopathy and increase in fungal diseases. Annual crops for such crop rotation targeting Cu phytoextraction include maize (*Zea mays* L.) and tobacco (*Nicotiana tabacum* L.) (Faessler et al. 2010a; Herzig et al. 2014), the last one having less water requirements. The Cu amount phytoextracted by maize, sunflower, and tobacco averaged 165, 270, and 285 g ha^{-1} $year^{-1}$, respectively, over the 6 years of crop rotation (Faessler et al. 2010a). Copper tolerance of some tobacco cultivars is reported (Keller et al. 2003; Kolbas et al. 2013) while tobacco species are potentially suitable for bioavailable Cd and Cu stripping in southwest (SW) France (Mench et al. 1989; Hattab et al. 2016) and Belgium (Vangronsveld et al. 2009; Thijs et al. 2018). Metal-resistant somaclonal variants of tobacco have been selected and tested in Switzerland and Belgium (Lyubenova et al. 2009; Vangronsveld et al. 2009; Herzig et al. 2014; Thijs et al. 2018). Compared with parental lines, the best variants had a higher shoot metal concentration, i.e., 5–7-fold for Cu, 2–5-fold for Cd, and 1.5-fold for Zn, in hydroponics and their antioxidant status differed (Guadagnini 2000; Lyubenova et al. 2009). Such tobacco variants are candidates for non-food crop rotation on metal-contaminated soils, but their productivity and shoot metal removal in SW Europe, notably at sites with Cu-contaminated soils, remained not documented.

Plant biomasses harvested at phytomanaged sites, notably tobacco shoots, can be processed by various technologies, e.g., pyrolysis, hydrothermal oxidation, fermentation, gasification, etc., to produce different compounds, e.g., biochar, hydrogen fuel, oil and glycerine, biofuel, bioethanol, bioplastic and activated carbons, Cu fertilizers, derived catalysts in syntheses of functionalized aromatic derivatives, etc. (Van Ginneken et al. 2007; Andrianov et al. 2010; Bohmert-Tatarev et al. 2011; Clavé et al. 2016; Gonsalvesh et al. 2016; Grisan et al. 2016; Poltronieri 2016; Asad et al. 2017; Huang et al. 2018; Yuan et al. 2019; Zhang et al. 2019a, b).

This study aimed at determining the phytoextraction capacity of one Cu-resistant tobacco variant and two tobacco clones by measuring their shoot DW yields and shoot Cu removals, depending on total soil Cu and soil treatments in field plots established at a wood preservation site in SW France. The questions were: (1) Does the somaclonal variant accumulate more Cu in shoots than both clones in field plots as in hydroponics? If yes, at which soil Cu exposure does this difference most expressed? (2) Are the phenotypic traits of tobacco variant and both clones changed as Cu exposure increases? (3) What is the Cu distribution in plant parts? (4) What is the influence of soil conditioners on plant biomass and shoot ionome?

Materials and methods

Site

The wood preservation site (10 ha), located in Saint Médard d'Eyrans, SW France (N 44°43.353, W 000°30.938), has been used for over a century (Mench and Bes 2009). Creosote, Cu sulfate (1913–1980), chromated copper arsenate type C (1980–2006), Cu hydroxycarbonates (17.3%) with benzylalkonium chloride (4.8%), and finally Tanalith E (Cu carbonate 16.4%, tebuconazole 0.18%, and propiconazole 0.18% *w/w*) were successively used (Mench et al. 2018). No preserved wood was stored on the field trial at least since 2003. Site characteristics are detailed in Mench and Bes (2009), Bes et al. (2010), and Kolbas et al. (2011).

Climatic conditions are typically oceanic. Air humidity and precipitations were low at the beginning of the 2010 growing season as compared to previous years (Fig. 1). Anthropogenic topsoils are developed on an alluvial sandy soil (Fluvisol–Eutric Gleysols, World Reference Base for soil resources, Table 1). Initial risk assessment and soil investigation pits (0–1.5 m) revealed a major Cu contamination in topsoils (0–25 cm) with spatial variation (65 to 2400 mg Cu kg^{-1} soil), which mirrored wood washings, whereas total soil As and Cr, i.e., 10–53 mg As and 20–87 mg Cr kg^{-1} in topsoils, were relatively low in all soil layers (Mench and Bes 2009; Kolbas et al. 2011) (Table 1).

The field trial (10 m × 11 m), located at the P1–3 sub-site, consisted in four blocks (2 m × 10 m) (Kolbas et al. 2011). In March 2008, the topsoil was loosened with a tiller (0–50 cm soil depth), the integrity of the deeper soil horizons being preserved. Compost made from poultry manure and pine bark chips (CPM, 5% *w/w*; Orisol, Cestas, France) and dolomitic limestone (DL, 0.2% *w/w*, containing 30% CaO and 20% MgO combined with carbonates, fineness index 80% < 0.16 mm, neutralizing power 58; Prodical Carmeuse, Orthez, France) were incorporated in April 2008 into topsoils of three blocks (henceforth referred to OMDL #1 to #3). Each amended block was divided in 10 plots (1 m × 2 m). One additional plot (#33, OMZ) was amended with 1% of zerovalent iron grit (Z, Bes and Mench 2008) and 5% of compost (CPM). The whole block #4 remained untreated (UNT, plot #31). An uncontaminated plot (#32, 1 m × 2 m, control soil, CTRL) with a similar soil type (Fluvisol) was also managed at a kitchen garden located 18 km from the site (Gradignan, France). Overall, 27 plots were cultivated with the tobacco plants in 2010, after growing sunflowers in 2008–2009, to assess a suitable crop rotation (Table 2). Topsoils and soil pore waters from all plots were collected and analyzed (Table 2) as described by Kolbas et al. (2011). In contaminated plots, total soil Cu (0–25 cm soil layer) exceeded the background values for French sandy soils, i.e., 3.2 mg and 8.4 mg Cu kg^{-1} for median and upper whisker

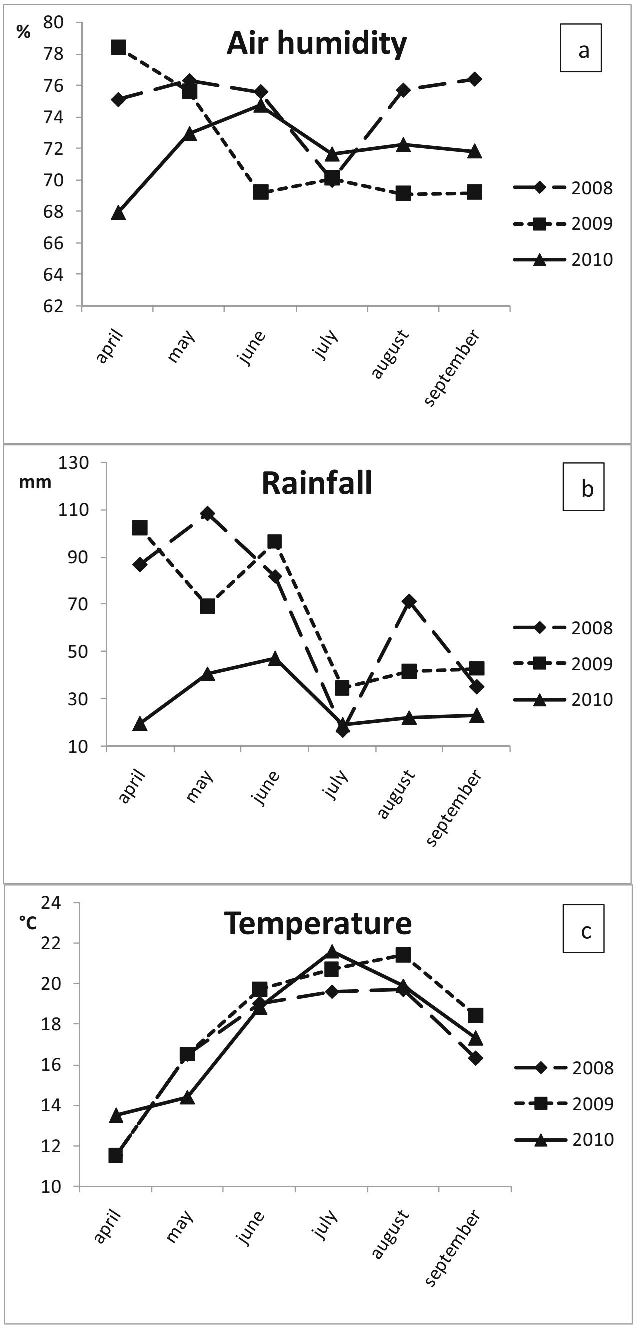

Fig. 1 Climatic conditions during the 2008–2010 period at the field trial: (**a**) monthly air humidity (%), (**b**) monthly rainfall (mm), and (**c**) monthly air temperature (°C)

values (Baize 1997), and for topsoils in Aquitaine region, France, i.e., 13.9 mg and 55.8 mg Cu kg^{-1} for median and upper whisker values (El Hadri et al. 2012).

Plants

One Cu-resistant somaclonal variant (C1—NBCu 10-8-F1 2009, derived from the BaG clone) and two metal-tolerant clones (C2—Forchheimer Pereg FoP-F1 2009; C3—Badischer Geudertheimer BaG-F1 2004) of tobacco (*Nicotiana tabacum* L.), henceforth referred to cultivars for convenience, were used (Guadagnini 2000; Lyubenova et al. 2009; Herzig et al. 2014). Tobacco seeds were sown (February 4, 2010) and seedlings were transplanted (March 5) in plastic pots (7 cm × 7 cm × 7 cm) containing a mixture of peat, sand, and control soil at growing stage 1000 (CORESTA 2019). Pots were placed in a greenhouse and then plants at growing stage 1003–1005 (CORESTA 2019) were transplanted in all field plots (April 14). In each plot, the plantation design consisted of three rows with four plants for each cultivar in a row, resulting potentially in 12 plants/plot/cultivar and a density of 180,000 plants ha^{-1}, which is higher than the common one for tobacco crop but permits to increase the overall biomass yield (Sheen 1983). Four additional C2 plants were transplanted at the plot #11 edge to study metal distribution in plant parts. Split applications of NPK fertilizer (NFU 42-001, 15-15-15) were made (40 kg N, 40 kg P_2O_5, 40 kg K_2O, and 60 kg SO_3 per ha on April 26 and 20 kg N, 20 kg P_2O_5, 20 kg K_2O, and 30 kg SO_3 on May 20). Irrigations (7.5 m^3 ha^{-1}) were made on April 14 and 26, May 28, and June 14 and 28, as these periods displayed abnormal drought in 2010 (Fig. 1).

Shoots were manually harvested (cut 1, July 19) at growth stage 9000 (CORESTA 2019). Stems were cut 1 cm above the soil surface and root systems let in place. For studying metal distribution in plant parts, roots, stems, leaves, and inflorescences (cymes with seeds in capsules) were separately collected for the four additional C2 plants (plot #11). Thereafter, bottom suckers began to develop, and their shoots were harvested on September 20 (cut 2). For both harvests, plant number per plot was recorded and shoot length and FW yield were measured for all plants. Roots were firstly washed with tap water. Plant parts were rinsed two times with distilled water, blotted with ash-free filter paper, air-dried (40–50 °C) in a greenhouse until constant weight, and weighed (DW). Water content (WC, %) in harvested plant parts was determined. Plant samples were grounded in a stainless mill (Gondard, 2 mm). Weighed aliquots of plant materials (0.5 g DW) were wet digested under microwaves (Marsxpress, CEM) with 5 mL supra-pure 14 M HNO_3 and 2 mL 30% (*v*/*v*) H_2O_2 not stabilized by phosphates. Certified reference material (BIPEA maize V463) and blank reagents were included in all series. Element concentrations in digests were determined by ICP-AES (Varian Liberty 200). All elements were recovered (> 95%) according to the standard values and standard deviation for replicates was < 5%.

Statistical analysis

All analyses were performed using the R software version 2.13.1 (Foundation for Statistical Computing, Vienna, Austria). A principal component analysis (PCA) was

Table 1 Main soil properties (0–0.25 m soil depth)

Soil		P1–3*	OMZ	UNT	CTRL	Soil survey in 1998	Background values in French sandy soils[a]	French screening values[a]	Soil quality criteria[c]	
									B	C
Sand	%	85.5	83	83.7	66.5					
Silt	%	8.3	14	10.9	15.5					
Clay	%	5.9	3	5.4	18.0	4.2				
C/N		17.2	17.4	15.9	13.8					
Organic C	g kg^{-1}	7.75	13.6	10.02	41.3					
OM	%	1.6	2.89	1.45	4.0	3.7				
CEC	cmol kg^{-1}	3.5	5.33	3.09	16.1					
pH		7.0	7.18	6.3	7.9					
As	mg kg^{-1}	9.8	9.83	7.31	3.6	10–22	1.0–25[b]		12	12
Cd	mg kg^{-1}	0.12	0.06				0.03–0.24	0.70	1.4/10	22
Co	mg kg^{-1}	< 2	4.86	1.90			1.4–6.8	30	40/50	300
Cu	mg kg^{-1}	**1460**	**1290**	**1016**	21	308–14,585	3.2–8.4	35	63	91
Cr	mg kg^{-1}	23	**90.7**	20.4	18	123–691	14.1–40.2	100	64	87
Fe	mg kg^{-1}	6090	35,725	6301	6550		6000–14,300			
Mn	mg kg^{-1}	181	394	160	189		72–376			
Ni	mg kg^{-1}	5	38.6	5.9	7.5		4.2–14.5	70	45	89
Pb	mg kg^{-1}	27	24	21	24		16.4–58.7	60	70/140	260/600
Tl	mg kg^{-1}	0.24					0.29		1	1
Zn	mg kg^{-1}	46	49.5	37.2	51		17–48	150	250	410
pCu^{2+}		7.66[d]	8.03	6.92	12.38					

[*] Sub-samples from the P1, P2, and P3 soils were combined to form the P1–3 soil sample—data from preliminary investigations in year 2006, before set up of field trial. [a] Median and high vibrissae values except for As (Baize 1997; Baize and Tercé 2002); [b] frequent As mean values for all French soil types (Baize and Tercé 2002); [c] B: agricultural/residential, C: commercial/industrial (Canadian Council of Ministers of the Environment 2020); [d] $pCu^{2+} = 3.20 + 1.47\ pH - 1.84\ \log_{10}$ (CuT) (Sauvé 2003). Bold value indicates a concentration in excess compared to background level

CTRL uncontaminated control soil, *OMZ* compost + zerovalent iron grit, *UNT* untreated soil

performed on both plant and soil parameters. Values were centered and scaled prior to analysis. A correlation coefficient matrix was then computed on the same parameter list using the Pearson coefficient Rp. Correlations were considered significant at $p < 0.05$. We tested the interactive effects of total soil Cu (CuT, covariate) and cultivar (three-level factor) on SL, SDWY, CuR, and CuSH using a mixed analysis of covariance (ANCOVA) model. Plots were included as a random variable in the model. We ran quadratic rather than linear models because parameter responses to CuT may display a hormesis shape (Calabrese and Blain 2009). Data were averaged per plot and cultivar prior to analysis of each plant parameters. All data were log transformed to meet parametric model assumptions (homoscedasticity and normality of residuals).

We tested the effects of amendments, Cu exposure, and cultivars on all plant parameters considered in the PCA analysis using two-way ANOVA models on non-averaged data taken from five plots consisting in five different conditions (amendment/Cu exposure factor). Differences between cultivars within each amendment/Cu exposure condition and between all amendment/Cu exposure conditions for each cultivar were tested using the Scott–Knott clustering algorithm. The same analysis was used to discriminate differences in biomass, Cu concentration, and Cu removal between the plant parts. Homoscedasticity and normality of residuals were met for all models.

Total soil Cu in amended blocks #1 and #2 were similar, so in further calculations, their data were merged as the OMDL #1 + 2 treatment with moderate total soil Cu, whereas data from the amended plots of block #3 with high total soil Cu were merged within the OMDL #3 treatment (Table 2). Distribution of shoot DW yield versus shoot Cu concentration was fitted using non-linear least squares estimates of the parameters of a Michaelis–Menten equation: $f(x) = a \times x/(p + x)$.

Results and discussion

All transplants survived and developed in all plots, without visible symptom on aerial parts. In comparison, sunflower mortality rate in the previous year varied from 0% to 85%

Table 2 Concentrations of metal(loid)s in topsoils of field plots and main properties of their soil pore waters

		Total soil concentrations (mg kg^{-1})						Soil pore water				
Block[a]	Plot number	Cu	Cr	Ni	Zn	Co	As	CuSPW mg Cu L^{-1}	[b] pCu^{2+}	Free Cu ratio (%)	pH in SPW	TOC in SPW mg C L^{-1}
OMDL #1	2	**273**	18.3	5.1	**98.4**	1.8	6.0	0.200	7.11	2.47	7.2	100.9
OMDL #1	3	**334**	19.2	4.8	42.9	1.7	6.5	0.264	7.41	0.93	7.2	112.1
OMDL #1	4	**384**	19.4	4.9	**48.4**	1.7	6.9	0.251	6.90	3.20	7.2	95.3
OMDL #1	5	**333**	19.3	5.1	**64.5**	2.1	6.6	0.248	7.20	1.62	7.4	124.0
OMDL #1	6	**239**	17.6	5.2	**59.4**	1.9	5.9	0.171	7.20	2.35	7.3	96.4
OMDL #1	7	**359**	17.8	5.0	**62.4**	1.9	6.7	0.258	7.12	1.89	7.3	145.5
OMDL #1	8	**268**	16.4	5.1	**58.1**	2.0	5.3	0.266	7.27	1.29	7.3	172.0
OMDL #1	9	**348**	21.1	5.3	**69.7**	1.8	8.6	0.188	7.01	3.28	7.2	76.0
OMDL #2	12	**336**	17.3	4.8	**80.8**	1.8	6.3	0.211	7.40	1.21	7.4	125.7
OMDL #2	13	**518**	19.4	4.8	**50.7**	1.8	6.9	0.206	7.31	1.53	7.3	92.1
OMDL #2	14	**382**	17.5	4.9	**50.1**	1.7	5.6	0.324	7.23	1.17	7.0	111.5
OMDL #2	15	**379**	19	5.1	**56.6**	1.9	6.9	0.219	7.39	1.18	7.0	97.6
OMDL #2	16	**317**	17.4	4.8	**51**	1.9	5.9	0.208	7.31	1.49	7.0	102.1
OMDL #2	17	**352**	20	5.2	**51.2**	1.9	7.1	0.256	7.21	1.53	7.1	104.3
OMDL #2	18	**357**	19.5	5.1	**52.3**	1.9	7.2	0.217	7.36	1.29	7.3	119.7
OMDL #2	19	**258**	18.2	5.5	**52.2**	2.0	6.2	0.247	7.35	1.15	7.0	93.4
OMDL #3	22	**1140**	18.4	5.3	45.4	1.8	6.7	0.785	6.23	4.84	7.1	110.0
OMDL #3	23	**819**	15.8	4.9	35.7	1.8	4.8	0.764	6.41	3.21	7.0	115.9
OMDL #3	24	**952**	16.5	4.9	41.7	1.7	5.0	0.795	6.21	4.91	6.9	123.6
OMDL #3	25	**961**	19.3	4.7	39.1	1.5	5.7	0.837	6.30	3.85	6.8	110.2
OMDL #3	26	**753**	16.2	5.0	39	1.8	5.4	0.776	6.35	3.70	6.7	136.0
OMDL #3	27	**1070**	17.1	5.4	**51.6**	1.9	5.9	0.590	6.48	3.58	6.9	120.0
OMDL #3	28	**894**	16.2	5.3	47.9	2.0	5.3	0.729	6.39	3.54	6.8	159.8
OMDL #3	29	**1020**	16.7	5.6	**53.8**	2.0	5.1	0.743	6.78	1.41	7.2	150.0
UNT	31	**1016**	18.8	6.0	35.2	1.8	5.7	0.547	5.38	49.08	5.9	43.8
CTRL	32	**21**	18	7.5	**51**	0.7	3.6	0.150	7.44	1.56	7.9	60.7
OMZ	33	**1290**	52.3	19.5	**57.6**	2.94	8.45	0.298	6.49	3.01	7.18	nd

[a] Plots #1 to #19 (blocks OMDL #1 + #2) correspond to moderate total soil Cu and plots #22 to #29 (block OMDL #3) correspond to high total soil Cu.

[b] pCu^{2+} = − log$_{10}$(Cu^{2+}), analyzed by Cu-ion selective electrode. Bold value indicates a concentration in excess compared to background level

nd not determined, *SPW* soil pore water, *TOC* total organic carbon, *CuSPW* total Cu concentration in the soil pore water, *CTRL* uncontaminated control soil, *OMDL* compost + dolomitic limestone, *OMZ* compost + zerovalent iron grit, *UNT* untreated soil

on the same plots and sunflowers did not survive in the UNT plot (Kolbas et al. 2011). Tobacco plants would be more tolerant but may benefit of transplantation rather than sowing and the small volume (9 cm^3) of clean soil attached to plantlet roots. Indeed, on a series of potted Cu-contaminated soils from the same site, these tobacco plants have demonstrated a higher Cu tolerance to excess Cu than sunflower plants (Kolbas et al. 2013).

Shoot yield versus soil Cu exposure and soil treatments

The PCA on soil and plant parameters explained 69% of the total variance (axe #1, 41%; axe #2, 28%) (Fig. 2) and showed several correlations listed in the correlation matrix (Table 3). Shoot growth traits (group 1—SDWY, SFWY, and SL) were highly correlated (Table 3). Shoot WC was not affected by Cu exposure. Soil parameters characterizing Cu exposure (group 2—CuT and CuSPW) co-varied similarly (0.92) and well correlated with shoot Cu concentration (0.81 and 0.76, respectively, Table 3). Relationship between the Log$_{10}$SL and Log$_{10}$CuT was well fitted by a curvilinear model for each cultivar with a hormesis effect (Calabrese and Blain 2009) around 400–500 mg Cu kg^{-1} soil ($p = 0.012$; Fig. 3a). The ANCOVA test indicated also a significant hormesis for SDWY ($p = 0.04$, Fig. 3b). Such field trial results confirmed the increase in shoot DW yield of FoP plants at moderate total soil Cu reported in a pot experiment (Kolbas et al. 2013).

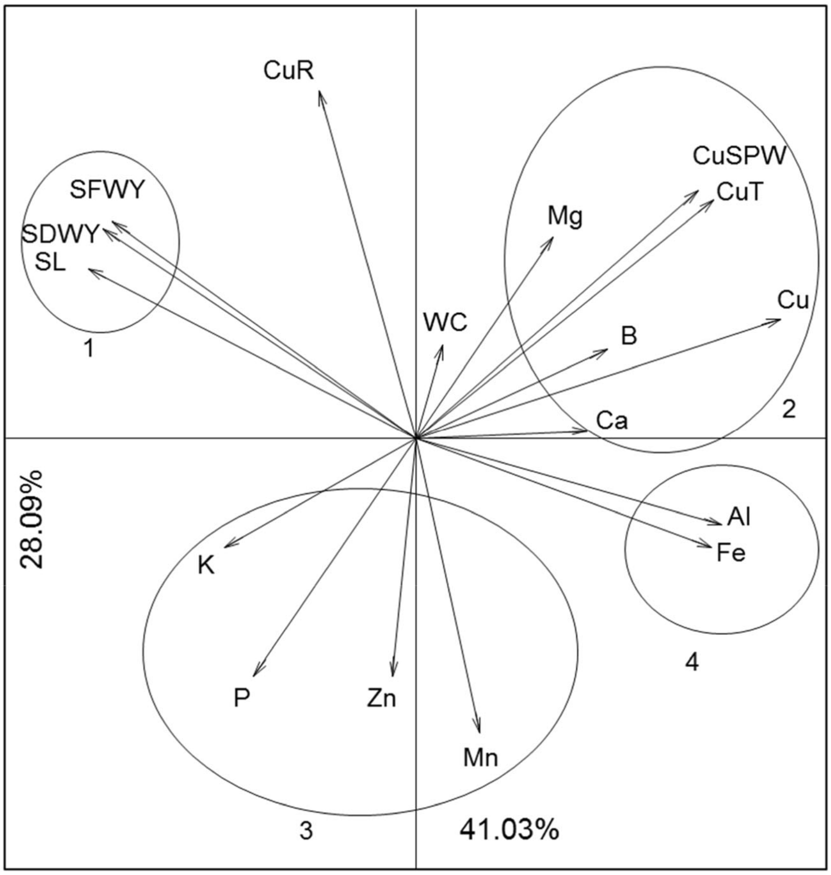

Fig. 2 PCA and correlations between plant parameters (SDWY, shoot DW yield; SFWY, shoot fresh weight yield; SL, shoot length; CuR, shoot Cu removal; and WC, water content of shoots), shoot element concentrations (Al, B, Ca, Cu, K, Fe, Mg, Mn, P, and Zn) and soil parameters of Cu exposure (CuSPW, total Cu concentration in the soil pore water; CuT, total soil). The numbers 1 to 4 correspond to groups of positively correlated parameters

Despite a high rise in total soil Cu and CuSPW, shoot DW yield only slightly decreased ($r = -0.31$ with CuT and -0.29 with CuSPW, Table 3), confirming the Cu resistance of these tobacco cultivars (Kolbas et al. 2013). On all plots, the SL and SDWY significantly differed between cultivars (ANCOVA test; $p < 0.01$), being in decreasing order: C2 > C1 > C3 (Fig. 3a, b). However, based on each plot, the SL and SDWY values only differed between cultivars for the OMDL #3 and OMZ plots (Table 4). The total SDWY (i.e., cuts 1 + 2 in t DW ha^{-1} year^{-1}) peaked for all cultivars in plot #12 (CuT—336 mg Cu kg^{-1} soil; CuSPW—0.21 mg Cu L^{-1}) up to 7.3, 9.9, and 5.3 for C1, C2, and C3, respectively. At high total soil Cu (753–1140 mg Cu kg^{-1}), SDWY peaked in plot #28 (i.e., 4.9, 3.1, and 2.4 t DW ha^{-1} year^{-1} for C1, C2, and C3, respectively). Plant growth in this plot was similar to that in the control soil (Table 4). Shoot DW yields of cut 2 ranged from 0.03 (plot #9) to 1.43 (plot #12) t DW ha^{-1} contributing between 9.9% (OMDL #1 + 2) and 43.1% (OMZ) to the total SDWY (Table 5). Higher SDWY of bottom suckers in the OMDL #3 plots may be due to higher amount of nutrients remaining in the soil after cut 1 and the lower growth of the main stem. In previous field trials, the SDWY of tobacco cultivated in the 15–525 mg Cu kg^{-1} soil range varied between 8 and 12.6 t DW ha^{-1} year^{-1} (Table 6). Unfavorable weather conditions (i.e., droughty spring and summer in 2010, Fig. 1), higher soil Cu contamination, and low soil fertility (Table 1) would explain lower shoot DW yields obtained in our field trial. Higher Cu tolerance and shoot yields of tobacco cultivars compared to sunflower in our Cu-contaminated plots may be related to their high antioxidant capacity. On a Zn/Cd/Pb-contaminated soil, tobacco plants originating from the FoP clone had high APX activities, while the NBCu variants (BaG origin) exhibited strong induction of the DHAR activity, and both BaG and FoP descendants displayed high SOD and CAT activities (Lyubenova et al. 2009).

Shoot Cu concentrations

Both total soil Cu (ANCOVA, $p < 0.001$, Fig. 2c) and cultivars (ANCOVA, $p = 0.02$, Table 4) influenced shoot Cu concentrations. After log transformation, CuSH linearly varied with total soil Cu (Fig. 2c). Roughly over 753 mg Cu kg^{-1} soil, shoot Cu concentrations of C1 and C2 plants were lower than those of C3 ones (Table 4), which may be due to their numerically higher SDWY values (i.e., dilution effect). Differences were less than in hydroponics (i.e., 5–7 times for Cu; Guadagnini 2000). Considered separately, shoot Cu concentrations may lead to misconception due to the dilution effect reducing their values as shoot biomass increased (Fig. 4). The SDWY and CuSH were negatively correlated ($R^2 = -0.49$;

Table 3 Pearson's correlation coefficients between soil and shoot parameters

	SL	SFWY	SDWY	WC	CuR	CuT	CuSPW	Al	B	Ca	Cu	Fe	Mg	Mn	P	K
SFWY	**0.9*****															
SDWY	**0.91*****	**0.97*****														
WC	0.17$^{\mathrm{NS}}$	0.26*	0.09$^{\mathrm{NS}}$													
CuR	**0.6*****	**0.69*****	**0.73*****	0.05$^{\mathrm{NS}}$												
CuT	−0.35**	−0.29**	−0.31**	0.17$^{\mathrm{NS}}$	0.26*											
CuSPW	−0.32**	−0.25*	−0.29**	0.25*	0.28*	**0.92*****										
Al	**−0.51*****	**−0.45*****	**−0.46*****	0.13$^{\mathrm{NS}}$	−0.19$^{\mathrm{NS}}$	0.35**	0.3**									
B	−0.31**	−0.2$^{\mathrm{NS}}$	−0.24*	0.11$^{\mathrm{NS}}$	0.02$^{\mathrm{NS}}$	0.31**	**0.39*****	**0.45*****								
Ca	−0.17$^{\mathrm{NS}}$	−0.04$^{\mathrm{NS}}$	−0.14$^{\mathrm{NS}}$	**0.52*****	−0.07$^{\mathrm{NS}}$	0.19 $^{\mathrm{NS}}$	0.22$^{\mathrm{NS}}$	**0.52*****	0.3**							
Cu	**−0.58*****	**−0.48*****	**−0.49*****	0.08$^{\mathrm{NS}}$	0.14$^{\mathrm{NS}}$	**0.81*****	**0.76*****	**0.62*****	0.37**	0.36**						
Fe	**−0.47*****	**−0.43*****	**−0.46*****	0.25*	−0.22*	0.35**	0.29**	**0.87*****	0.14$^{\mathrm{NS}}$	**0.64*****	**0.64*****					
Mg	−0.19$^{\mathrm{NS}}$	−0.08$^{\mathrm{NS}}$	−0.15$^{\mathrm{NS}}$	0.28*	0.22*	**0.49*****	**0.65*****	−0.02$^{\mathrm{NS}}$	0.52***	0.21$^{\mathrm{NS}}$	**0.37*****	−0.09$^{\mathrm{NS}}$				
Mn	−0.25*	−0.36**	−0.33**	−0.22*	**−0.36*****	−0.22*	−0.21$^{\mathrm{NS}}$	0.29**	−0.16$^{\mathrm{NS}}$	0.04$^{\mathrm{NS}}$	0.04$^{\mathrm{NS}}$	**0.39*****	−0.29**			
P	0.06$^{\mathrm{NS}}$	0.04$^{\mathrm{NS}}$	−0.01$^{\mathrm{NS}}$	0.14$^{\mathrm{NS}}$	**−0.4*****	**−0.58*****	**−0.44*****	−0.22$^{\mathrm{NS}}$	0.01$^{\mathrm{NS}}$	−0.05$^{\mathrm{NS}}$	**−0.54*****	−0.22*	0.1$^{\mathrm{NS}}$	0.23*		
K	0.31**	0.31**	0.22$^{\mathrm{NS}}$	0.43***	−0.16$^{\mathrm{NS}}$	**−0.45*****	−0.31**	−0.27*	−0.07$^{\mathrm{NS}}$	0.19$^{\mathrm{NS}}$	**− 0.51*****	−0.22*	0.15$^{\mathrm{NS}}$	0.06$^{\mathrm{NS}}$	**0.79*****	
Zn	−0.04$^{\mathrm{NS}}$	−0.04$^{\mathrm{NS}}$	−0.03$^{\mathrm{NS}}$	0.02$^{\mathrm{NS}}$	−0.15$^{\mathrm{NS}}$	−0.26*	−0.29**	0.25*	−0.15$^{\mathrm{NS}}$	0.05$^{\mathrm{NS}}$	−0.07$^{\mathrm{NS}}$	0.3**	−0.31**	**0.5*****	**0.41*****	0.24**

$^{\mathrm{NS}}$Not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$

SL shoot length, *SDWY* shoot dry weight yield, *SFWY* shoot fresh weight yield, *WC* water content of shoots, *CuR* shoot Cu removal, *CuT* total soil Cu, *CuSPW* total Cu concentration in the soil pore water, shoot element concentrations: Al, B, Ca, Cu, Fe, Mg, Mn, P, K, and Zn

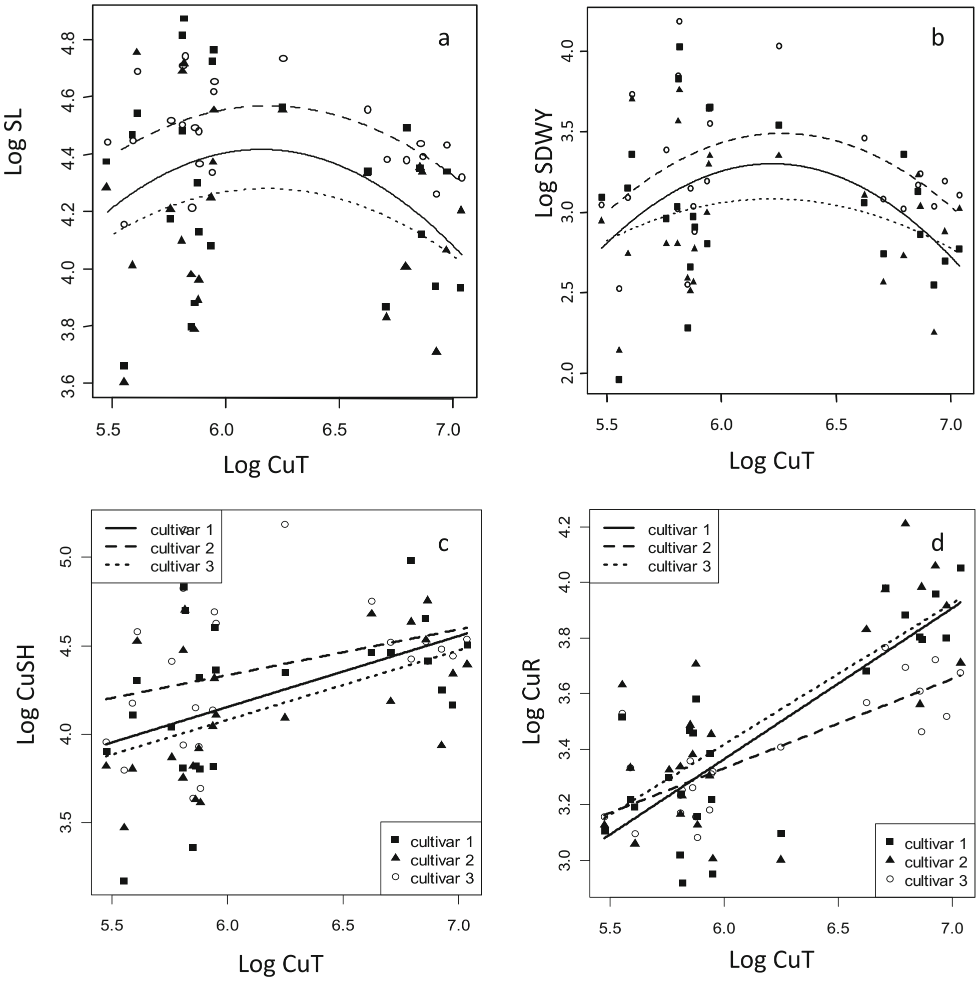

Fig. 3 Relationships between plant growth parameters and total soil Cu (CuT) (all data log transformed): (**a**) SL—stem length, (**b**) SDWY—shoot DW yield, (**c**) CuSH—shoot Cu concentration, and (**d**) CuR—shoot Cu removal

$p < 0.001$; Table 3). Based on all cultivars, the relationship between SDWY and CuSH values was fitted using non-linear least squares estimates of the parameters of a Michaelis–Menten equation, i.e., SDWY = 1.32 CuSH/(CuSH − 13.01) ($R^2 = 0.62$; $p < 0.01$), which indicated an EC_{50} value in the 28–32 mg Cu kg^{-1} shoot DW range (Fig. 4). This agreed with upper critical Cu threshold values in shoots (Ali et al. 2002; Macnicol and Beckett 1985; Mocquot et al. 1996). Copper mainly accumulates in intracellular structures such as the vacuoles and therefore the number of cells is related with Cu concentrations (Printz et al. 2016). On the whole dataset, mean shoot Cu concentration was 33 mg Cu kg^{-1} DW and varied from 6 mg (C2 in the CTRL plot) to 75 mg kg^{-1} (C2 in the OMZ plot; Table 4). In the literature, shoot Cu concentrations reported for tobacco grown in field trials ranged from 5 to 90 mg kg^{-1} (Table 6).

Shoot Cu removal

Shoot Cu removal (g Cu ha^{-1} year^{-1}) was computed with shoot Cu concentration (mg Cu kg^{-1} DW) and shoot biomass (t ha^{-1} year^{-1}) (Table 4, Fig. 3d). Assuming similar shoot Cu concentrations for both cuts, additional shoot Cu removal by the bottom suckers would be in the 7.2–73.5 g Cu ha^{-1} year^{-1} range and consequently, annual shoot Cu removal varied between 15.4 and 261.3 g Cu ha^{-1} year^{-1}, depending on cultivars and soil treatments (Table 4). The CuR value differed between cultivars (ANCOVA, $p = 0.0011$, Fig. 3d), displaying highest values for the C1 plants as Cu exposure increased in the OMDL #3 plots (Table 4). These values were significantly higher than those for sunflowers on the same OMDL #3 plots in 2008 (10–58 g Cu ha^{-1} year^{-1}; Kolbas et al. 2011) and in 2016 (47–70 g Cu ha^{-1} year^{-1}; Mench et al. 2018), but were slightly lower than previous findings for tobacco (~280 g Cu ha^{-1} year^{-1}; Kayser et al. 2000; Faessler et al. 2010a) (Table 6). As highlighted by the PCA (Fig. 2), shoot Cu removal was more correlated with plant growth parameters (group 1), in particular shoot DW yield, than with shoot Cu concentration (Table 3). In contrast to sunflower, CuT and CuSPW less influenced shoot Cu removal by tobacco ($r = 0.26$ and $r = 0.28$, respectively), one difference being the growth of bottom suckers after cut 1. However, following years have demonstrated that this depends more and more on water supply and summer climatic conditions, in line with

Table 4 Shoot growth parameters, shoot ionome (cut 1), and shoot Cu removals depending on tobacco cultivars and soil treatments

Soil treatment	Cultivar	SL (cut 1) cm	SDWY (cut 1) t DW ha^{-1} $year^{-1}$	SDWY (cuts 1 + 2) t DW ha^{-1} $year^{-1}$	CuR (cut 1) g Cu ha^{-1} $year^{-1}$	CuR (cuts 1 + 2) g Cu ha^{-1} $year^{-1}$	Cu (cut 1) mg kg^{-1}	Al (cut 1) mg kg^{-1}	B (cut 1) mg kg^{-1}	Ca (cut 1) g kg^{-1}	Fe (cut 1) mg kg^{-1}	Mg (cut 1) g kg^{-1}	Mn (cut 1) mg kg^{-1}	P (cut 1) g kg^{-1}	K (cut 1) g kg^{-1}	Zn (cut 1) mg kg^{-1}
CTRL	C1	91.9a[*]B[**]	3.6aB	5.8aB	26.4aB	43.2aC	7.5aC	85.2aC	35.3aA	17.2aA	157.7aC	3.8aA	30.4aC	3.9aA	49.9aA	26.2aB
	C2	86.0aB	2.2aB	2.6bB	13.3aC	15.4bC	6.0aD	83.9aD	27.1aB	16.7aA	132.7aB	3.4aA	26.1aC	3.2bA	49.7aA	16.3bB
	C3	88.4aB	2.6aB	2.9bB	21.3aB	24.1bC	8.2aD	92.9aC	33.2aA	15.9aA	168.0aC	3.5aA	25.7aC	3.8aA	42.7aA	26.8aA
OMDL #1 + 2	C1	130.3aA	5.9aA	7.3bA	109.6bA	134.7bB	18.5aC	146.8aB	25.6aA	10.4aB	179.3aC	2.6aB	40.2aB	2.5aB	28.8aB	31.7aB
	C2	114.5aA	6.9aA	9.9aA	173.3aA	255.9aA	25.9aC	119.8bD	20.8aB	10.7aB	155.4aB	2.5aB	43.5aB	2.4aB	29.2aB	30.0aA
	C3	111.5aA	4.5bA	5.7cA	110.2bA	143.2bA	25.3aC	172.3aB	25.3aA	10.2aB	205.1aB	2.3aB	38.6aB	2.3aB	24.5aB	28.7aA
OMDL #3	C1	88.8aC	3.0aB	4.9aB	145.7aA	235.9aA	48.6bB	196.7aB	39.6aA	11.3aB	191.8aC	3.9aA	28.9aC	2.3aB	26.8aB	25.9aB
	C2	79.6aB	2.2aB	3.1bB	83.5bB	124.6bB	40.2bB	160.3aC	26.0aB	11.1aB	184.1aB	3.6aA	28.3aC	2.0aB	26.2aB	24.6aA
	C3	54.8bC	1.6aC	2.4bB	100.0bA	163.1bA	67.5aA	175.6aB	30.7aA	11.4aB	218.6aB	3.5aA	25.2aC	1.9aB	23.7a	21.9aB
OMZ	C1	36.3bC	0.8aC	1.5aC	40.4aB	105aB	70.2aA	203.7bB	31.8bA	12.1bB	289.9bB	1.9bC	27.7bC	1.2aC	12.6aC	16.8bC
	C2	59.3aC	1.1aB	1.6aC	64.9aB	120aB	75.2aA	743.2aA	45.2aA	20.3aA	1041.6aA	2.6aB	47.6aB	1.4aC	14.6aC	29.4aA
	C3	40.3bC	0.8aC	1.2aC	28.5aB	61bB	50.5bB	157.2cB	26.7bA	13.6bB	214.4bB	1.9bB	32.8bB	1.2aC	15.5aC	16.7bB
UNT	C1	30.0aC	0.5aC	0.8aD	23.2bB	41.7bC	51.2aB	715.8aA	26.8aA	17.7aA	1137.8aA	2.4aB	67.9aA	2.2aB	25.7a	45.1aA
	C2	52.1aC	1.0aB	1.5aC	64.1aB	97.9aB	65.4bA	592.9bB	21.2aB	18.8aA	1182.3aA	2.3aB	72.9aA	1.5bC	26.4aB	35.0bA
	C3	36.4aC	0.7aC	1.1aC	23.8bB	40.2bC	36.1cC	446.7cA	19.5aA	11.7bB	694.1bA	2.2aB	46.6bA	2.1aB	28.5aB	36.2bA

[*] In each column and for the same soil treatment, mean values followed by the same lowercase letter did not differ between cultivars at the 5% level; [**] in a column and for each cultivar, mean values followed by the same uppercase letter did not differ between soil treatments at the 5% level

SL shoot length, *SDWY* shoot dry weight yield, *CuR* shoot Cu removal

Soil treatments: CTRL, uncontaminated control soil; OMDL, compost + dolomitic limestone; OMZ, compost + zerovalent iron grit; UNT, untreated soil

OMDL #1 + 2: OMDL plots of blocks 1 and 2; OMDL #3: OMDL plots of block 3

Table 5 Shoot growth parameters, shoot Cu removal, and shoot ionome of tobacco plants depending on soil treatments

	Shoot growth parameters					Shoot Cu removal			Shoot ionome									
Soil treatments	SL	SDWY (cut 1)	SDWY (cut 2)	SDWY (cuts 1 + 2)	Suckers[4]	CuR[1] (cut 1)	CuR[2] (cut 2)	CuR[3] (cuts 1 + 2)	Al (cut 1)	B (cut 1)	Ca (cut 1)	Cu (cut 1)	Fe (cut 1)	Mg (cut 1)	Mn (cut 1)	P (cut 1)	K (cut 1)	Zn (cut 1)
	cm	t DW $year^{-1}$	t DW $year^{-1}$	t DW $year^{-1}$	%	g ha^{-1} $year^{-1}$	g ha^{-1} $year^{-1}$	g ha^{-1} $year^{-1}$	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	g kg^{-1}	mg kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}
CTRL	88.8b	2.81b	0.95b	3.76b	25.3b	20.3c	6.9d	27.2d	87.3	31.9	16.6	7.2d	153	3.55	27.4	3.65	47.5	23.1
OMDL #1 + 2	188.8a	5.75a	0.63c	6.38a	9.9c	131.0a	14.5cd	145.5a	146.3	23.9	10.4	23.2c	180	2.45	40.8	2.40	27.5	30.1
OMDL #3	74.4c	2.26b	1.43a	3.69b	38.8a	109.7b	73.5a	183.2a	177.5	32.1	11.3	51.3b	198	3.67	27.5	2.07	25.6	24.1
OMZ	45.3d	0.90c	0.53c	1.43c	43.1a	44.6c	34.9b	79.5b	368.0	34.5	15.3	65.4a	515	2.10	36.0	1.27	14.2	21.0
UNT	39.5d	0.72c	0.42c	1.14c	36.8a	37.0c	21.4c	58.4c	585.2	22.5	16.1	50.9b	1005	2.28	62.5	1.95	26.9	38.8
Kabata-Pendias 2010	–	–	–		–	–	–	–	8–3400	10–100	13–24	8–15	50–250	4–8	50–120	2.5–4.5	25–45	25–70
Faessler et al. 2010a	–	–	–		–	–	–	–	–	–	22.8	18.6	76.2	2.7	17.9	2.01	27.4	63.1

[1] cut 1; [2] cut 2 (bottom suckers); [3] total—same letter did not differ between soil treatments at the 5% level; [4] contribution of bottom suckers to the annual shoot DW yield

SL shoot length, *SDWY* shoot dry weight yield, *CuR* shoot Cu removal

Soil treatments: CTRL, uncontaminated control soil; OMDL, compost + dolomitic limestone; OMZ, compost + zerovalent iron grit; UNT, untreated soil

OMDL #1 + 2: OMDL plots of blocks 1 and 2; OMDL #3: OMDL plots of block 3

progressive global warming. Shoot Cu removal opposed to group 3 and slightly negatively correlated with Mn and P ($r = -0.36$ and -0.4, respectively) (Fig. 2; Table 3).

Shoot concentrations and removals for other elements

Tobacco plants growing in contaminated soils generally display disturbances in nutritional status, specifically in Mg and P contents (Faessler et al. 2010a). Here, the shoot ionome showed significant dilution effects due to changes in SDWY for Al and Fe as for Cu (Fig. 2, Table 3). Shoot Al, Fe, Mg, Ca, and B concentrations positively correlated with shoot Cu concentration, and shoot P and K concentrations negatively (Table 3). Both cellular Ca and Mg homeostasis can help to quench oxidative stress in Cu-stressed plants (Kinraide et al. 2004), whereas B involved in carbohydrate transport has a synergetic relationship with Ca in tobacco (Lopez-Lefebre et al. 2001). Free Cu^{+} in the cytosol induces ROS generation thereby opening non-selective cationic channels, allowing the Ca^{2+} entry and inducing root growth (Printz et al. 2016). Shoot K, P, Zn, and Mn concentrations (group 3) co-varied and were opposed to soil Cu exposure and shoot Cu concentration (group 2, Fig. 2, Table 3). Such elements are essential for plant nutrition. Their antagonism with Cu concentrations in soil and shoots confirmed previous findings for old leaves of Cu-exposed *Elsholtzia splendens* (Wu et al. 2009) and may reflect impaired Cu-stressed roots causing ion (e.g., K^{+}) and solute leakages (Woolhouse and Walker 1981) and a malfunction of root transporters (Printz et al. 2016). A crosstalk is suggested between Cu and Zn absorption, but how Cu excess mediates with ZIP transporter expression remains unclear (Printz et al. 2016). Total soil Zn slightly exceeded its background levels for French sandy soils in several amended plots, in line with compost addition (Table 2). However, mean values for shoot Zn concentrations (24–39 mg Zn kg^{-1} DW) and shoot Zn removal (118–290 g Zn ha^{-1} $year^{-1}$) were within the lower published ones (Tables 5 and 6).

Aluminum and Fe (group 4) co-varied and negatively correlated with group 1 (Fig. 2). The mutual synergy between both elements were reported in the flue-cured tobacco nutrition (Chang et al. 1999). Aluminum has an oxidative potential in plant tissues and enhances the Fe-mediated lipid peroxidation, notably in phospholipid liposomes, which alters the permeability of the plasma membrane and leads to cell death (Yamamoto 2019). Positive relationships between shoot Al, Fe, and Cu concentrations, mainly driven by plant growth and soil treatments (Table 3), agreed with Fe/Cu correlation in maize (Ali et al. 2002), although it is usually negative for other plants (Palmer and Guerinot 2009). Shoot Cu, Al, and Fe concentrations peaked in the UNT and OMZ plants having low shoot biomass (Table 4) and exceeded their common values in tobacco shoots (Table 5).

Table 6 Copper and zinc concentrations in tobacco plant parts and shoot Cu and Zn removals in field trials

Total soil Cu[a]	Study type	Shoots Cu	Roots Cu	Stem Cu	Leaves Cu	Inflorescence Cu	Shoot DW yield	Cu R	Shoot Zn	Zn R	References
mg kg^{-1}**	P, H, F*	mg Cu kg^{-1} DW	mg Cu kg^{-1} DW	mg Cu kg^{-1} DW	mg Cu kg^{-1} DW	mg Cu kg^{-1} DW	t DW ha^{-1} year^{-1}	g Cu ha^{-1} year^{-1}	mg Zn kg^{-1} DW	kg Zn ha^{-1} year^{-1}	
CTRL	F		2.62	3.34	7.42	4.88			0.5–25.4		Ionescu et al. 2008
90	F		18	13	15.5–18.3	17			51–63		Zaprjanova and Bozhinova 2009
63 (15–399)	F	21–30	12.6–52.2	9.04–29.2	3.6–58.9	17.5–45.5			8.5–139		Zaprjanova et al. 2011
CTRL	F	5–10							18–60		Campbell 2000
0–450 with EDDS, EDTA	P	10–219	30–1400								Evangelou et al. 2007
(28–469)	F	60–90					11	285	9–72		Faessler et al. 2010a
27–54	P				3–10				10–170 (62–505 in soil)		Keller et al. 2005
(18–525)	F	38	26				12.6	280	146	1.844	Kayser et al. 2000
Zn–220	H						8 (24.7–37.5)		293–617	11–12	Vangronsveld et al. 2009
(239–518)	F	20–41	73.7	13.1	33.2	27.6	9.9	261	30.1	0.29	This study
(753–1290)	F	31–67.5					4.9	236	24.1	0.12	This study
	F								19.7–57.6	0.07	Gondola and Kadar 1995
0.1–100 μM Cu in solution	H	14–96							25–3427 (10–1000 μM Zn in solution)		Guadagnini 2000
6–126 μM Cu with NTA	H	1.5–200	10–3000								Wenger et al. 2003
43–48	P	5–110							20–1800 (149–1434)		Sappin-Didier et al. 1997

[*] P (pot or greenhouse), H (hydroponics), F (field); **for total soil Cu in pot and field experiments; [a] mean value (minimum and maximum values)

CTRL uncontaminated control soil, *EDDS* ethylenediamine-*N*,*N*′-disuccinic acid, *EDTA* ethylenediaminetetraacetic acid, *NTA* nitrilotriacetate, *CuR* Cu removal by aerial plant parts, *ZnR* Zn removal by aerial plant parts

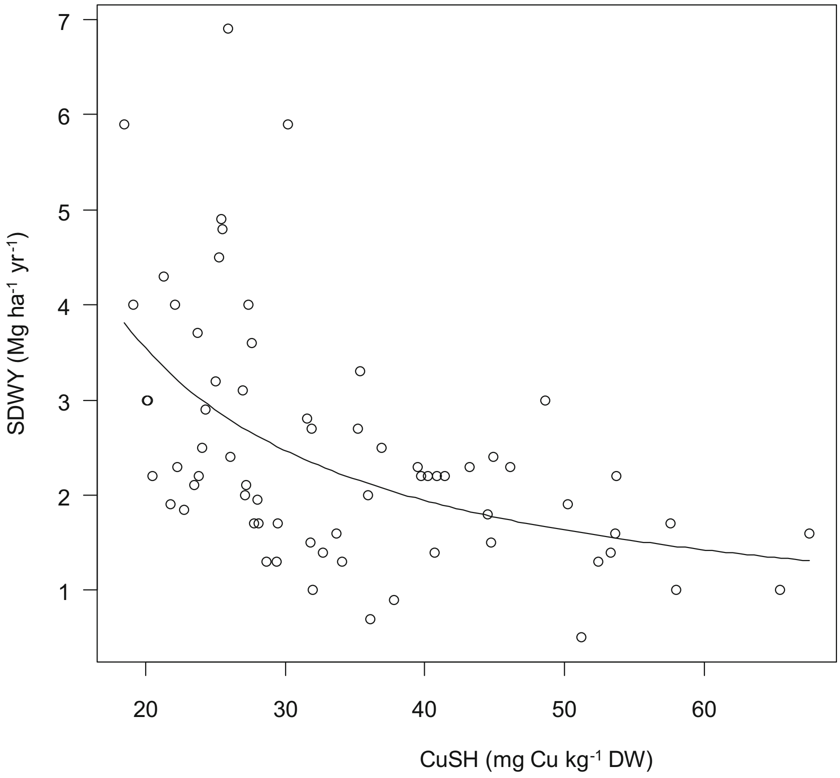

Fig. 4 Relationship between shoot DW yield (SDWY) and shoot Cu concentration (CuSH)

Influence of soil amendments on shoot ionome

Compost and dolomitic limestone incorporated into Cu-contaminated soils of this wood preservation site enhance plant growth, promote microbial activity, and reduce Cu leaching from the root zone (Bes and Mench 2008; Lagomarsino et al. 2011; Marchand et al. 2011; Mench et al. 2018; Burges et al. 2020). In the OMZ soil, Z caused a major shift in Cu distribution to the fraction bound to poorly crystalline Fe oxyhydroxides (Kumpiene et al. 2011), but this did not promote shoot DW yield nor induce a decreased shoot Cu concentration for the OMZ plants. The soil conditioners, however, influenced labile Cu pool in soils (Table 2), biometrical traits of tobacco (Fig. 3a, b), and shoot ionome of cultivars (Table 4), confirming that tobacco is responsive to improving amendments (Faessler et al. 2010b; Lyubenova et al. 2009). At similar high CuT values (1016–1290 mg Cu kg^{-1} soil), the UNT, OMZ, and OMDL #3 (plot #29) plants significantly differed for SL, SDWY, CuR, and shoot ionome (Table 5). While CuSH was slightly higher in the OMZ plants, the SDWY and CuR of OMDL #3 plants were higher. The UNT and OMZ plants had similar low SDWY values at both cuts, but the CuSH of OMZ plants was 1.3-fold higher which increased their shoot Cu removal (Table 5). In the OMZ and UNT soils, the C2 plants showed higher total CuR (120 and 97 g Cu ha^{-1} year^{-1}) than the C1 and C3 plants (Table 4). Conversely, total SDWY and CuR were higher for the C1 plants in OMDL #3.

The UNT plants had low shoot P concentrations but high shoot Cu, Al, and Fe concentrations. Similar trends occurred for the OMZ plants, with increased shoot Cu concentrations due to the composition of zerovalent iron grit (Kumpiene et al. 2011). Shoot concentrations of other elements were in the common ranges (Table 5). Shoot ionome did not differ between OMDL #1 + 2 and OMDL #3, except for Cu (Table 5). Shoot Cu concentration of the OMDL #3, UNT, and OMZ plants exceeded its upper critical threshold value (28–32 mg Cu kg^{-1}) (Table 4). Adding compost in the OMDL soils led to Cu binding with organic matter, particularly the coarse compost fraction (Lagomarsino et al. 2011), whereas liming may likely promote both this binding and Cu precipitation and sorption to (hydr)oxides and clays (Rachou et al. 2007). The lower Cu^{2+} ion concentration in the soil pore water of OMDL #3 soils than that of UNT soil (Table 2) was only reflected in shoot Cu concentration for the C2 plants (Table 4).

Biomass, Cu concentration, and Cu amount of plant parts

Aerial parts (stem, leaves, and inflorescence) represented 80% of the total biomass of FoP plants, the stem having the highest DW yield (Fig. 5a). Roots had a lower biomass than stem but the highest Cu concentration (Fig. 5b). Consequently, Cu amount peaked in roots (Fig. 5c), corresponding to an excluder strategy which may partly include Cu sorption on the root surface Fe/Mn plaque (Ye et al. 2001). Copper concentrations

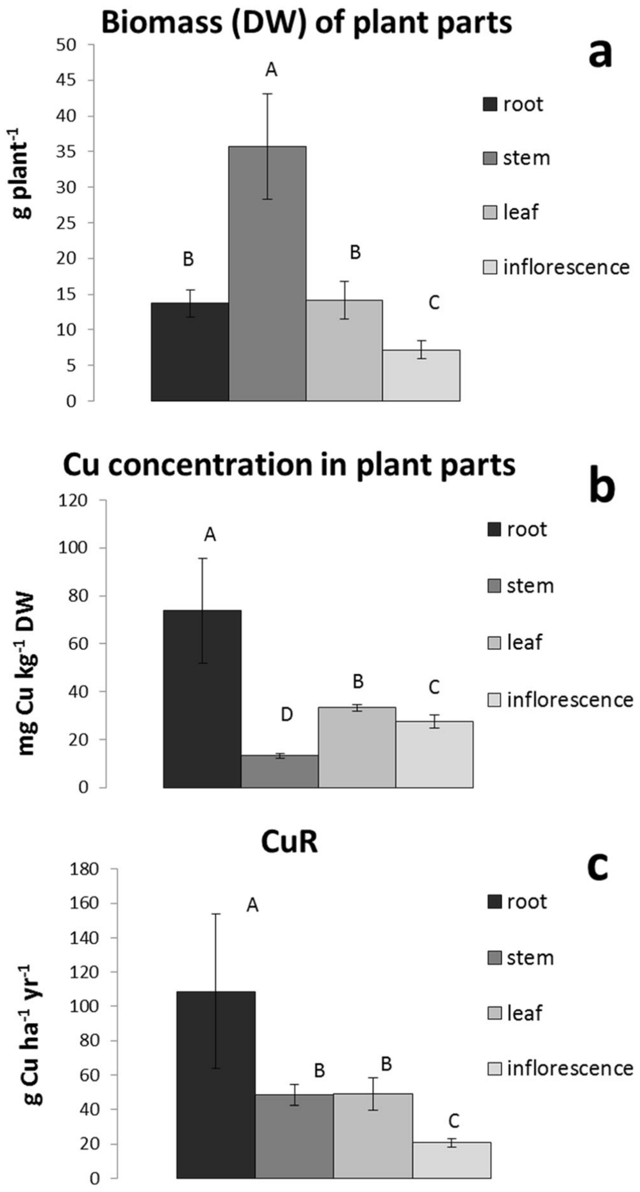

Fig. 5 (**a**) biomass (dry weight), (**b**) Cu concentration, and (**c**) Cu removal (CuR) of plant parts for the FoP-F1 tobacco cultivated in plot #11 (total soil Cu = 285 mg kg^{-1})

in the FoP tobacco parts were in decreasing order: roots > leaves > inflorescence (including seeds) > stem (Fig. 5b), whereas Cu amounts followed the decreasing order: roots > stem = leaves > inflorescence (Fig. 5c). Distributions of contaminants and roots in soil layers affect plant establishment and their phytoextraction ability (Keller et al. 2003). In our case, soil Cu contamination was mainly located between 0.2 and 0.7 m and, based on visual observations, roots remained in the 0–30 cm soil layer. Low Cu concentration in inflorescences including seeds, which contained up to 40% oil per DW, enables their use for biofuel production (Grisan et al. 2016; Poltronieri 2016). Tobacco leaves contain 1.7–4% oil per DW (Koiwai et al. 1983), which is extractable as fatty acid methyl esters, major components for biodiesel quality (García-Martínez et al. 2017). Based on a maximum SDWY of 9.8 t ha^{-1} year^{-1} and seed yield of 13% (1.27 t ha^{-1} year^{-1}), the oil production may reach 0.84 t ha^{-1} year^{-1}. The fermentation of tobacco biomass for producing bioethanol is also feasible (Olofsson et al. 2008). Bio-based muconic acid, a versatile chemical intermediate whose derivatives, i.e., caprolactam, terephthalic acid (a precursor to polyethylene terephthalate, a common thermoplastic polymer resin of the polyester family), and adipic acid, are widely used in the plastic industry, the production of synthetic fibers for textiles or industry (mainly nylon), and food (acidifying agent), can be produced from tobacco using specific bacteria (Eudes et al. 2018).

Phenotypic responses obtained for shoot Cu concentration and other tobacco parameters, i.e., SL, SDWY, and CuR, may reflect the parental proximity between the NBCu variant and the BaG clone (Guadagnini 2000). As the FoP clone resulted in higher shoot Cu removal at moderate soil Cu exposure, its somaclonal variants may deserve more investigations.

Conclusion

Merging aided phytoextraction and sustainable production of non-food crops is a developing strategy for phytomanaging Cu-contaminated soils. A Cu-resistant tobacco variant and two tobacco clones were cultivated in field plots with increasing total soil Cu and three soil treatments at a wood preservation site in SW France. Thanks to climatic conditions and cultural practices, shoots of main stem were firstly harvested (cut 1) and then shoots of bottom suckers (cut 2). Both shoot Cu concentration and Cu removal responded to changes in soil Cu exposure and shoot DW yields. Shoot Cu removal ranged from 13 to 173 g Cu ha^{-1} for cut 1 and annual shoot Cu removal may reach 15–261 g Cu ha^{-1} year^{-1} assuming similar shoot Cu concentrations in cut 2. At high total soil Cu, the incorporation of compost and dolomitic limestone (OMDL) into the soil was more efficient than compost with zerovalent iron grit (OMZ) to increase shoot Cu removal. Such soil conditioners allowed obtaining similar shoot yield at high total soil Cu compared to the uncontaminated control soil and higher shoot yield at moderate soil Cu exposure. Shoot Cu removal (cut 1) peaked for the FoP plants in the OMDL plots with moderate soil Cu contamination and the untreated plot. The NBCu variant phytoextracted more Cu than the BaG and FoP clones in the OMDL plots with high soil Cu contamination. Copper concentrations in the FoP plant parts were in decreasing order: roots > leaves > inflorescence > stem, and Cu removals ranked as: roots > stem = leaves > inflorescence. Shoot K, P, Mn, and Zn concentrations were negatively affected by Cu excess. As shoot DW yield mainly influenced shoot Cu removal, the selection of tobacco cultivars for Cu phytoextraction through in vitro breeding should account for both Cu concentrations in aerial plant parts and potential shoot production for financial returns.

Acknowledgments Researches at this contaminated site were supported by ADEME (French Energy and Environment Agency), Department of Urban Brownfields and Polluted Sites, Angers, France, the European Commission under the Seventh Framework Programme for Research (FP7-KBBE-266124, GREENLAND), the ERA-Net FACCE SURPLUS (project INTENSE; https://projects.au.dk/faccesurplus/research-projects-1st-call/intense/), and the PhytoSUDOE project (Interreg SUDOE SOE1/P5/E0189, http://www.phytosudoe.eu/). Authors thank Dmitry Trofimenko, Amélie Aztiria, and Dr. Nadège Oustriere for their technical assistance. The UMR Biogeco is a member of the INRAE Ecotoxicologist network, ECOTOX (https://www6.inrae.fr/ecotox_eng/), and the Labex COTE (https://cote.labex.u-bordeaux.fr/en/). This work is dedicated to the memory of our colleague Dr. Petra Susan Kidd, CSIC, Santiago de Compostela, Spain, one of the shining stars having developed phytotechnologies for phytomanaging contaminated sites.

References

Abbaszadeh-Dahaji P, Baniasad-Asgari A, Hamidpour M (2019) The effect of Cu-resistant plant growth-promoting rhizobacteria and EDTA on phytoremediation efficiency of plants in a Cu-contaminated soil. Environ Sci Pollut Res 26:31822–31833. https://doi.org/10.1007/s11356-019-06334-0

Ali NA, Bernal MP, Ater M (2002) Tolerance and bioaccumulation of copper in *Phragmites australis* and *Zea mays*. Plant Soil 239:103–111. https://doi.org/10.1023/A:1014995321560

Andrianov V, Borisjuk N, Pogrebnyak N, Brinker A, Dixon J, Spitsin S, Flynn J, Matyszczuk P, Andryszak K, Laurelli M, Golovkin M, Koprowski H (2010) Tobacco as a production platform for biofuel: overexpression of Arabidopsis DGAT and LEC2 genes increases accumulation and shifts the composition of lipids in green biomass. Plant Biotechnol J 8:277–287. https://doi.org/10.1111/j.1467-7652.2009.00458.x

Antoniadis V, Shaheen SM, Levizou E, Shahid M, Niazi NK, Vithanage M, Ok YS, Bolan N, Rinklebe J (2019) A critical prospective analysis of the potential toxicity of trace element regulation limits in soils worldwide: are they protective concerning health risk assessment?—A review. Environ Int 127:819–847. https://doi.org/10.1016/j.envint.2019.03.039

Asad M, Menana Z, Ziegler-Devin I, Bert V, Chalot M, Herzig R, Mench M, Brosse N (2017) Pretreatment of trace element-enriched biomasses grown on phytomanaged soils for bioethanol production. Ind Crop Prod 107:63–72. https://doi.org/10.1016/j.indcrop.2017.05.028

Baize (1997) Teneurs totales en éléments traces métalliques dans les sols (France), INRA Editions, Paris, France. 410 p. ISBN13 978–2–7380-0747-6

Baize D, Tercé M (2002) Les éléments traces métalliques dans les sols—Approches fonctionnelles et spatiales, INRA Editions (QUAE), Paris, France. 570 p. EAN13 9782759202355. ISBN 273800993X 9782738009937

Ballabio C, Panagos P, Lugato E, Huang JH, Orgiazzi A, Jones A, Fernández-Ugalde O, Borrelli P, Montanarella L (2018) Copper distribution in European topsoils: an assessment based on LUCAS soil survey. Sci Total Environ 636:282–298. https://doi.org/10.1016/j.scitotenv.2016.05.115

Benizri E, Kidd PS (2018) The role of the rhizosphere and microbes associated with hyperaccumulator plants in metal accumulation. In: Van der Ent A, Echevarria G, Baker AJM, Morel JL (eds) Agromining: farming for metals: extracting unconventional resources using plants. Book Series: Mineral Resource Reviews. Springer International Publishing AG, Cham, pp 157–188. https://doi.org/10.1007/978-3-319-61899-9_9

Bes C, Mench M (2008) Remediation of copper-contaminated topsoils from a wood treatment facility using in situ stabilisation. Environ Pollut 156:1128–1138. https://doi.org/10.1016/j.envpol.2008.04.006

Bes CM, Mench M, Aulen M, Gaste H, Taberly J (2010) Spatial variation of plant communities and shoot Cu concentrations of plant species at a timber treatment site. Plant Soil 330:267–280. https://doi.org/10.1007/s11104-009-0198-4

Bohmert-Tatarev K, McAvoy S, Daughtry S, Peoples OP, Snell KD (2011) High levels of bioplastic are produced in fertile transplastomic tobacco plants engineered with a synthetic operon for the production of polyhydroxybutyrate. Plant Physiol 155:1690–1708. https://doi.org/10.1104/pp.110.169581

Burges A, Fievet V, Oustriere N, Epelde L, Garbisu C, Becerril JM, Mench M (2020) Long-term phytomanagement with compost and a sunflower–tobacco rotation influences the structural microbial diversity of a Cu-contaminated soil. Sci Total Environ 700:134529. https://doi.org/10.1016/j.scitotenv.2019.134529

Calabrese EJ, Blain RB (2009) Hormesis and plant biology. Environ Pollut 157:42–48. https://doi.org/10.1016/j.envpol.2008.07.028

Campbell C (2000) Reference sufficiency ranges for plant analysis in the southern region of the United States. https://ag.tennessee.edu/spp/Documents/scsb394.pdf. Accessed on 10 January 2020

Canadian Council of Ministers of the Environment (2020) Canadian environmental quality guidelines. http://st-ts.ccme.ca/en/index.html. Accessed on 10 January 2020

Carcamo V, Bustamante E, Trangolao E, De la Fuente LM, Mench M, Neaman A, Ginocchio R (2012) Simultaneous in situ immobilization of metals and arsenic in acidic polluted soils near a copper smelter in central Chile. Environ Sci Pollut Res 19:1131–1143. https://doi.org/10.1007/s11356-011-0673-3

Cevher-Keskin B, Yildizhan Y, Yuksel B, Dalyan E, Memon AR (2019) Characterization of differentially expressed genes to Cu stress in *Brassica nigra* by *Arabidopsis* genome arrays. Environ Sci Pollut Res 26:299–311. https://doi.org/10.1007/s11356-018-3577-7

Chang YC, Yamamoto Y, Matsumoto H (1999) Accumulation of aluminium in the cell wall pectin in cultured tobacco (*Nicotiana tabacum* L.) cells treated with a combination of aluminium and iron. Plant Cell Environ 22:1009–1017. https://doi.org/10.1046/j.1365-3040.1999.00467.x

Clavé G, Garoux L, Boulanger C, Hesemann P, Grison C (2016) Ecological recycling of a bio-based catalyst for Cu Click reaction: a new strategy for a greener sustainable catalysis. https://doi.org/10.1002/slct.201600430

Cordova S, Neaman A, Gonzalez I, Ginocchio R, Fine P (2011) The effect of lime and compost amendments on the potential for the revegetation of metal-polluted, acidic soils. Geoderma 166:135–144. https://doi.org/10.1016/j.geoderma.2011.07.022

CORESTA (2019) A scale for coding growth stages in tobacco crops. Coresta guide n°7. https://www.coresta.org/sites/default/files/technical_documents/main/Guide-No07-Growth-Stages_Dec19.pdf. Accessed on 9 January 2020

Cundy A, Bardos P, Puschenreiter M, Mench M, Bert V, Friesl-Hanl W, Müller I, Li X, Weyens N, Witters N, Vangronsveld J (2016) Brownfields to green fields: realising wider benefits from practical contaminant phytomanagement strategies. J Environ Manag 184:67–77. https://doi.org/10.1016/j.jenvman.2016.03.028

El Hadri H, Chery P, Jalabert S, Lee A, Potin-Gautier M, Lespes G (2012) Assessment of diffuse contamination of agricultural soil by copper in Aquitaine region by using French national databases. Sci Total

Environ 441:239–247. https://doi.org/10.1016/j.scitotenv.2012.09.070

Eudes A, Berthomieu R, Hao Z, Zhao N, Teixeira Benitesa V, Baidoo EEK, Loqué D (2018) Production of muconic acid in plants. Metab Eng 46:13–19. https://doi.org/10.1016/j.ymben.2018.02.002

Evangelou MWH, Bauer U, Ebel M, Schaeffer A (2007) The influence of EDDS and EDTA on the uptake of heavy metals of Cd and Cu from soil with tobacco *Nicotiana tabacum*. Chemosphere 68:345–353 https://doi.org/10.1016/j.chemosphere.2006.12.058

Faessler E, Robinson BH, Stauffer W, Gupta SK, Papritz A, Schulin R (2010a) Phytomanagement of metal-contaminated agricultural land using sunflower, maize and tobacco. Agric Ecosyst Environ 136:49–58. https://doi.org/10.1016/j.agee.2009.11.007

Faessler E, Robinson BH, Gupta SK, Schulin R (2010b) Uptake and allocation of plant nutrients and Cd in maize, sunflower and tobacco growing on contaminated soil and the effect of soil conditioners under field conditions. Nutr Cycl Agroecosyst 87:339–352. https://doi.org/10.1007/s10705-009-9342-z

Frick H, Tardif S, Kandeler E, Holm PE, Brandt KK (2019) Assessment of biochar and zero-valent iron for in-situ remediation of chromated copper arsenate contaminated soil. Sci Total Environ 655:414–422. https://doi.org/10.1016/j.scitotenv.2018.11.193

García-Martínez N, Andreo-Martínez P, Quesada-Medina J, Pérez de los Ríos A, Chica A, Beneito-Ruiz R, Carratalá-Abril J (2017) Optimization of non-catalytic transesterification of tobacco (*Nicotiana tabacum*) seed oil using supercritical methanol to biodiesel production. Energ Convers Manag 131:99–108. https://doi.org/10.1016/j.enconman.2016.10.078

Ginocchio R, Carvallo G, Toro I, Bustamante E, Silva Y, Sepulveda N (2004) Micro-spatial variation of soil metal pollution and plant recruitment near a copper smelter in Central Chile. Environ Pollut 127:343–352. https://doi.org/10.1016/j.envpol.2003.08.020

Gondola I, Kadar I (1995) Heavy metal content of flue-cured tobacco leaf in different growing regions of Hungary. Acta Agron Hung 43:243–251

Gonsalvesh L, Yperman J, Carleer R, Mench M, Herzig R, Vangronsveld J (2016) Valorisation of heavy metals enriched tobacco biomass through slow pyrolysis and steam activation. J Chem Technol Biotechnol 91:1585–1595. https://doi.org/10.1002/jctb.4889

Grisan S, Polizzotto R, Raiola P, Cristiani S, Ventura F, di Lucia F, Zuin M, Tommasini S, Morbidelli R, Damiani F, Pupilli F, Bellucci M (2016) Alternative use of tobacco as a sustainable crop for seed oil, biofuel, and biomass. Agron Sustain Dev 36:55. https://doi.org/10.1007/s13593-016-0395-5

Guadagnini M (2000) In vitro-breeding for metal-accumulation in two tobacco (*Nicotiana tabacum*) cultivars [PhD Dissertation]: University of Freiburg, Nr. 1288, Switzerland

Hattab N, Motelica-Heino M, Mench M (2016) Aided phytoextraction of Cu, Pb, Zn, and As in copper-contaminated soils with tobacco and sunflower in crop rotation: mobility and phytoavailability assessment. Chemosphere 145:543–550. https://doi.org/10.1016/j.chemosphere.2015.11.051

Herzig R, Nehnevajova E, Pfistner C, Schwitzguebel JP, Ricci A, Keller C (2014) Feasibility of labile Zn phytoextraction using enhanced tobacco and sunflower: results of five- and one-year field-scale experiments in Switzerland. Int J Phytoremediation 16:735–754. https://doi.org/10.1080/15226514.2013.856846

Huang F, Li WZ, Liu QC, Zhang TW, An SX, Li DW, Zhu XF (2018) Sulfonated tobacco stem carbon as efficient catalyst for dehydration of C6 carbohydrate to 5-hydroxymethylfurfural in γ-valerolactone/water. Fuel Process Technol 181:294–303. https://doi.org/10.1016/j.fuproc.2018.09.026

Ionescu AD, Burzo I, Ionescu OS (2008) Variance of the mineral content from different organs of two Virginia tobacco cultivars. Lucrari Stiintifice—Universitatea de Stiinte Agronomice si Medicina Veterinara Bucuresti. Seria B, Horticultura, 583–585. http://horticulturejournal.usamv.ro/pdf/7./Lucrari/stiintifice,/Seria/B,/Horticultura,/Vol/LI,/2008/(CD-ROM).pdf. Accessed on 15 January 2020

Kabata-Pendias A (2010) Trace elements in soils and plants, 4th edn. CRC Press Inc., Boca Raton ISBN 9781420093681

Kayser A, Wenger K, Keller A, Attinger W, Felix HR, Gupta SK, Schulin R (2000) Enhancement of phytoextraction of Zn, Cd, and Cu from calcareous soil: the use of NTA and sulfur amendments. Environ Sci Technol 34:1778–1783. https://doi.org/10.1021/es990697s

Keller C, Hammer D, Kayser A, Richner W, Brodbeck M, Sennhauser M (2003) Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field. Plant Soil 249:67–81. https://doi.org/10.1023/A:1022590609042

Keller C, Marchetti M, Rossi L, Lugon-Moulin N (2005) Reduction of cadmium availability to tobacco (*Nicotiana tabacum*) plants using soil amendments in low cadmium-contaminated agricultural soils: a pot experiment. Plant Soil 276:69–84. https://doi.org/10.1007/s11104-005-3101-y

Kidd P, Mench M, Álvarez-López V, Bert V, Dimitriou I, Friesl-Hanl W, Herzig R, Janssen JO, Kolbas A, Müller I, Neu S, Renella G, Ruttens A, Vangronsveld J, Puschenreiter M (2015) Agronomic practices for improving gentle remediation of trace-element-contaminated soils. Int J Phytorem 17:1005–1037. https://doi.org/10.1080/15226514.2014.1003788

Kinraide TB, Pedler JF, Parker DR (2004) Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH. Plant Soil 259:201–208. https://doi.org/10.1023/B:PLSO.0000020972.18777.99

Koiwai A, Suzuki F, Matsuzaki T, Kawashima N (1983) The fatty-acid composition of seeds and leaves of *Nicotiana* species. Phytochemistry 22:1409–1412. https://doi.org/10.1016/S0031-9422(00)84024-8

Kolbas A, Mench M, Herzig R, Nehnevajova E, Bes CM (2011) Copper phytoextraction in tandem with oilseed production using commercial cultivars and mutant lines of sunflower. Int J Phytorem 13(Suppl 1):55–76. https://doi.org/10.1080/15226514.2011.568536

Kolbas A, Mench M, Kolbas N, Marchand L, Herzig R (2013) Morphological and physiological responses of sunflower and tobacco grown on a Cu-contaminated soil series. 12th Int Conf on the Biogeochemistry of Trace Elements, 16–20 June, Athens, USA. pp 78–79

Kumpiene J, Mench M, Bes CM, Fitts JP (2011) Assessment of aided phytostabilization of copper-contaminated soil by X-ray absorption spectroscopy and chemical extractions. Environ Pollut 159:1536–1542. https://doi.org/10.1016/j.envpol.2011.03.005

Kumpiene J, Antelo J, Brannvall E, Carabante I, Ek K, Komarek M, Soderberg C, Warell L (2019) In situ chemical stabilization of trace element-contaminated soil—field demonstrations and barriers to transition from laboratory to the field—a review. Appl Geochem 100:335–351. https://doi.org/10.1016/j.apgeochem.2018.12.003

Lagomarsino A, Mench M, Marabottini R, Pignataro A, Grego S, Renella G, Stazi SR (2011) Copper distribution and hydrolase activities in a contaminated soil amended with dolomitic limestone and compost. Ecotoxicol Environ Saf 74:2013–2019. https://doi.org/10.1016/j.ecoenv.2011.06.013

Li JT, Gurajala HK, Wu LH, van der Ent A, Qiu RL, Baker AJM, Tang YT, Yang XE, Shu WS (2018) Hyperaccumulator plants from China: a synthesis of the current state of knowledge. Environ Sci Technol 52:11980–11994. https://doi.org/10.1021/acs.est.8b01060

Lillo-Robles F, Tapia-Gatica J, Diaz-Siefer P, Moya H, Youlton C, Celis-Diez JL, Santa-Cruz J, Ginocchio R, Sauve S, Brykov VA, Neaman A (2019) Which soil Cu pool governs phytotoxicity in field-collected soils contaminated by copper smelting activities in central Chile? Chemosphere 242:125176. https://doi.org/10.1016/j.chemosphere.2019.125176

Lopez-Lefebre LR, Rivero RM, Garcia PC, Sanchez E, Ruiz JM, Romero L (2001) Effect of calcium on mineral nutrient uptake and growth of tobacco. J Sci Food Agric 81:1334–1338. https://doi.org/10.1002/jsfa.948

Lyubenova L, Nehnevajova E, Herzig R, Schroeder P (2009) Response of antioxidant enzymes in *Nicotiana tabacum* clones during phytoextraction of heavy metals. Environ Sci Pollut Res 16:573–581. https://doi.org/10.1007/s11356-009-0175-8

Macnicol RD, Beckett PHT (1985) Critical tissue concentrations of potentially toxic elements. Plant Soil 85:107–129. https://doi.org/10.1007/BF02197805

Maderova L, Watson M, Paton GI (2011) Bioavailability and toxicity of copper in soils: integrating chemical approaches with responses of microbial biosensors. Soil Biol Biochem 43:1162–1168. https://doi.org/10.1016/j.soilbio.2011.02.004

Marchand L, Mench M, Marchand C, Le Coustumer P, Kolbas A, Maalouf J-P (2011) Phytotoxicity testing of lysimeter leachates from aided phytostabilized Cu-contaminated soils using duckweed (*Lemna minor* L.). Sci Total Environ 410:146–153. https://doi.org/10.1016/j.scitotenv.2011.09.049

Meers E, Van Slycken S, Adriaensen K, Ruttens A, Vangronsveld J, Du Laing G, Witters N, Thewys T, Tack FMG (2010) The use of bioenergy crops (*Zea mays*) for ‘phytoattenuation’ of heavy metals on moderately contaminated soils: a field experiment. Chemosphere 78:35–41. https://doi.org/10.1016/j.chemosphere.2009.08.015

Mench M, Bes C (2009) Assessment of ecotoxicity of topsoils from a wood treatment site. Pedosphere 19:143–155. https://doi.org/10.1016/S1002-0160(09)60104-1

Mench M, Tancogne J, Gomez A, Juste C (1989) Cadmium bioavailability to *Nicotiana tabacum* L., *Nicotiana rustica* L., and *Zea mays* L. grown in soil amended or not amended with cadmium nitrate. Biol Fertil Soils 8:48–53. https://doi.org/10.1007/BF00260515

Mench M, Lepp N, Bert V, Schwitzguebel J-P, Gawronski SW, Schroeder P, Vangronsveld J (2010a) Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. J Soils Sediments 10:1039–1070. https://doi.org/10.1007/s11368-010-0190-x

Mench M, Bes C M, Jaunatre R, Hego E, Kolbas A, Gasté H, Aulen M, Taberly J, Guinberteau J, Kechit F, San Martin-Garcia S, Bedon F, Chaumeil P, Lalanne C, Plomion C, Negim O, Gawronski S, Gawronska H, Kucharski R, Sas-Nowosielska A, Van der Lelie D, Vangronsveld J, Herzig R, Nehnevajova E, Palagi P M, Barré A, Claverol S, Dupuy JW, Bonneu M, Kumpiene J, Touzard B, Laporte-Cru J (2010b) Phytoremédiation d’un site et de sols contaminés par des produits de traitement du bois. Final Report, convention Ademe 05 72 C0018/INRA 22000033, UMR BIOGECO INRA 1202, Talence, 461 p

Mench M, Oustrière N, Marchand L, Dellise M, Poschenrieder Ch (2017) Phytomanagement of a Cu-contaminated soil with *Erucastrum incanum* (L.) W.D.J. Koch (*Hirschfeldia incana* (L.) Lagr.-Foss). The 14th International Phytotechnologies Conference, September 25–29, Montréal, Canada

Mench M, Dellise M, Bes CM, Marchand L, Kolbas A, Le Coustumer P, Oustrière N (2018) Phytomanagement and remediation of Cu-contaminated soils by high yielding crops at a former wood preservation site: sunflower biomass and ionome. Front Ecol Evol 6:123. https://doi.org/10.3389/fevo.2018.00123

Mocquot B, Vangronsveld J, Clijsters H, Mench M (1996) Copper toxicity in young maize (*Zea mays* L.) plants: effects on growth, mineral and chlorophyll contents, and enzyme activities. Plant Soil 182:287–300. https://doi.org/10.1007/BF00029060

Nowack B, Schulin R, Robinson BH (2006) Critical assessment of chelant-enhanced metal phytoextraction. Environ Sci Technol 40:5225–5232. https://doi.org/10.1021/es0604919

Olofsson K, Bertilsson M, Liden G (2008) A short review on SSF—an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels 1:7. https://doi.org/10.1186/1754-6834-1-7

Palmer CM, Guerinot ML (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nat Chem Biol 5:333–340. https://doi.org/10.1038/nchembio.166

Pardo J, Mondaca P, Celis-Diez JL, Ginocchio R, Navarro-Villarroel C, Neaman A (2018) Assessment of revegetation of an acidic metal(-loid)-polluted soils six years after the incorporation of lime with and without compost. Geoderma 331:81–86. https://doi.org/10.1016/j.geoderma.2018.06.018

Poltronieri P (2016) Tobacco seed oil for biofuels. In: Poltronieri P, D'Urso OF (eds) Biotransformation of agricultural waste and by-products. The food, feed, fibre, fuel (4F) economy. Elsevier, Amsterdam, pp 161–187. https://doi.org/10.1016/B978-0-12-803622-8.00006-9

Printz B, Lutts S, Hausman JF, Sergeant K (2016) Copper trafficking in plants and its implication on cell wall dynamics. Front Plant Sci 7:601. https://doi.org/10.3389/fpls.2016.00601

Rachou J, Gagnon C, Sauve S (2007) Use of an ion-selective electrode for free copper measurements in low salinity and low ionic strength matrices. Environ Chem 4:90–97. https://doi.org/10.1071/EN06036

Rehman M, Liu LJ, Wang Q, Saleem MH, Bashir S, Ullah S, Peng DX (2019) Copper environmental toxicology, recent advances, and future outlook: a review. Environ Sci Pollut Res 26:18003–18016. https://doi.org/10.1007/s11356-019-05073-6

Ren XM, Guo SJ, Tian W, Chen Y, Han H, Chen E, Li BL, Li YY, Chen ZJ (2019) Effects of plant growth-promoting bacteria (PGPB) inoculation on the growth, antioxidant activity, Cu uptake, and bacterial community structure of rape (*Brassica napus* L.) grown in Cu-contaminated agricultural soil. Front Microbiol 10:1455. https://doi.org/10.3389/fmicb.2019.01455

Sappin-Didier VL, Mench MJ, Gomez AN, Lambrot C (1997) Use of inorganic amendments for reducing metal bioavailability to ryegrass and tobacco in contaminated soils. In: Iskandar IK, Adriano DC (eds) Advances in Environmental Science. Science Reviews, Northwood, pp 85–98

Sauvé S (2003) Modelling trace element exposure and effects on plants. In: Mench M, Mocquot B (eds) Risk assessment and sustainable land management using plants in trace element-contaminated soils. Centre INRA Bordeaux-Aquitaine, Villenave d’Ornon, France. pp 69–70. https://www.researchgate.net/publication/337717040_Risk_assessment_and_sustainable_land_management_using_plants_in_trace_element-contaminated_soils_COST_Action_837_4th_WG2_Workshop_Bordeaux'2002. Accessed 9 January 2020

Shahbaz M, Pilon M (2019) Conserved Cu-microRNAs in *Arabidopsis thaliana* function in copper economy under deficiency. Plants-Basel 8:141. https://doi.org/10.3390/plants8060141

Sheen SJ (1983) Biomass and chemical-composition of tobacco plants under high-density growth. Beitr Tabakforsch Int 12:35–42. https://doi.org/10.2478/cttr-2013-0523

Tardif S, Cipullo S, Sø HU, Wragg J, Holm PE, Coulon F, Brandt KK, Cave M (2019) Factors governing the solid phase distribution of Cr, Cu and As in contaminated soil after 40 years of ageing. Sci Total Environ 652:744–754. https://doi.org/10.1016/j.scitotenv.2018.10.244

Taylor AA, Tsuji JS, Garry MR, McArdle ME, Goodfellow WL Jr, Adams WJ, Menzie CA (2020) Critical review of exposure and effects: implications for setting regulatory health criteria for ingested copper. Environ Manag 65:131–159. https://doi.org/10.1007/s00267-019-01234-y

Thijs S, Witters N, Janssen J, Ruttens A, Weyens N, Herzig R, Mench MJ, Van Slycken S, Meers E, Meiresonne L, Vangronsveld J (2018) Tobacco, sunflower and high biomass SRC clones show potential for trace metal phytoextraction on a moderately contaminated field site in Belgium. Front Plant Sci 9:1879. https://doi.org/10.3389/fpls.2018.01879

Tóth G, Hermann T, Szatmári G, Pásztor L (2016) Maps of heavy metals in the soils of the European Union and proposed priority areas for detailed assessment. Sci Total Environ 565:1054–1062. https://doi.org/10.1016/j.scitotenv.2016.05.115

Van Ginneken L, Meers E, Guisson R, Ruttens A, Elst K, Tack FMG, Vangronsveld J, Diels L, Dejonghe W (2007) Phytoremediation for heavy metal-contaminated soils combined with bioenergy production. J Environ Eng Landsc Manag 15:227–236. https://doi.org/10.1080/16486897.2007.9636935

Vangronsveld J, Herzig R, Weyens N, Boulet J, Adriaensen K, Ruttens A, Thewys T, Vassilev A, Meers E, Nehnevajova E, van der Lelie D, Mench M (2009) Phytoremediation of contaminated soils and groundwater: lessons from the field. Environ Sci Pollut Res 16:765–794. https://doi.org/10.1007/s11356-009-0213-6

Villanneau E, Perry-Giraud C, Saby N, Jolivet C, Marot F, Maton D, Floch-Barneaud A, Antoni V, Arrouays D (2008) Détection de valeurs anomaliques d'éléments traces métalliques dans les sols à l'aide du Réseau de Mesure de la Qualité des Sols. Etude et Gestion des Sols 15:183–200 https://www.afes.fr/wp-content/uploads/2017/10/EGS_15_3_villanneau.pdf. Accessed on 15 January 2020

Wenger K, Gupta SK, Furrer G, Schulin R (2003) The role of nitrilotriacetate in copper uptake by tobacco. J Environ Qual 32:1669–1676. https://doi.org/10.2134/jeq2003.1669

Witters N, Mendelsohn RO, Slycken SV, Weyens N, Schreurs E, Meers E, Tack F, Carleer R, Vangronsveld J (2012) Phytoremediation, a sustainable remediation technology? Conclusions from a case study. I: Energy production and carbon dioxide abatement. Biomass Bioenergy 39:454–469. https://doi.org/10.1016/j.biombioe.2011.08.016

Woolhouse HW, Walker S (1981) The physiological basis of copper toxicity and copper tolerance in higher plants. In: Loneragan JF, Robson AD, Graham RD (eds) Copper in soils and plants. Academic Press, Inc, New York, pp 235–262

Wu B, Chen Y, Becker JS (2009) Study of essential element accumulation in the leaves of a Cu-tolerant plant *Elsholtzia splendens* after Cu treatment by imaging laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS). Anal Chim Acta 633:165–172. https://doi.org/10.1016/j.aca.2008.11.052

Xue K, Zhou J, Van Nostrand JD, Mench M, Bes C, Giagnoni L, Arenella M, Renella G (2018) Functional activity and functional gene diversity of a Cu-contaminated soil remediated by aided phytostabilization using compost, dolomitic limestone and a mixed tree stand. Environ Pollut 242:229–238. https://doi.org/10.1016/j.envpol.2018.06.057

Yamamoto Y (2019) Aluminum toxicity in plant cells: mechanisms of cell death and inhibition of cell elongation. Soil Sci Plant Nutr 65:41–55. https://doi.org/10.1080/00380768.2018.1553484

Ye ZH, Cheung KC, Wong MH (2001) Copper uptake in *Typha latifolia* as affected by iron and manganese plaque on the root surface. Can J Bot 79:314–320. https://doi.org/10.1139/b01-012

Yruela I (2009) Copper in plants: acquisition, transport and interactions. Funct Plant Biol 36:409–430. https://doi.org/10.1071/FP08288

Yuan Y, Zou P, Zhou JH, Geng YT, Fan JJ, Clark J, Li YQ, Zhang CS (2019) Microwave-assisted hydrothermal extraction of non-structural carbohydrates and hemicelluloses from tobacco biomass. Carbohydr Polym 223:115043. https://doi.org/10.1016/j.carbpol.2019.115043

Zaprjanova P, Bozhinova R (2009) Nutritional element and heavy metal content in plant organs of the oriental and Virginia tobacco. Ecol Future J Agric Sci For Sci 8:20–29 ISSN: 1312–0751

Zaprjanova PS, Ivanov KI, Angelova VR, Dospatliev LK (2011) Relation between soil characteristics and copper and zinc content in Virginia tobacco. In Conference on the Biogeochemistry of Trace Elements (11th ICOBTE), Florence, Italy. pp 645–646

Zhang J, Zhang JY, Wang M, Wu SC, Wang H, Niazi NK, Man YB, Christie P, Shan SD, Wong MH (2019a) Effect of tobacco stem-derived biochar on soil metal immobilization and the cultivation of tobacco plant. J Soils Sediments 19:2313. https://doi.org/10.1007/s11368-018-02226-x

Zhang Y, Xu PF, Liang S, Liu BX, Shuai Y, Li BX (2019b) Exergy analysis of hydrogen production from steam gasification of biomass: a review. Int J Hydrog Energy 44:14290–14302. https://doi.org/10.1016/j.ijhydene.2019.02.064