

Treatment of an Acid Soil with Bentonite Used for Wine Fining: Effects on Soil Properties and the Growth of *Lolium multiflorum*

MANUEL ARIAS-ESTÉVEZ,[†] EUGENIO LÓPEZ-PERIAGO,[†] JUAN C. NÓVOA-MUÑOZ,[†]
 ANA TORRADO-AGRASAR,[‡] AND JESUS SIMAL-GÁNDARA^{*,§}

Soil and Agricultural Science Group, Plant Biology and Soil Science Department, Chemical Engineering Department, and Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology, University of Vigo, Ourense Campus, E32004 Ourense, Spain

When used to fine wines, bentonite acquires a protein load that makes it a potentially useful fertilizer. Other properties of bentonite are also potentially useful for soil amendment. In the work described in this paper, waste bentonite from a winery was applied to an acid soil, and its effects on soil properties and on the growth of *Lolium multiflorum* were evaluated. Soil N, K, and P contents all increased, as did pH and cation exchange capacity. Biomass production increased as the dose of bentonite increased up to 5 g kg⁻¹, decreasing at larger doses (possibly as a result of falling potassium/magnesium ratio and increasing electrical conductivity). Environmental drawbacks of waste bentonite include its high soluble copper content, although its conversion in the soil to less soluble forms reduces its potential phytotoxicity. The copper, manganese, and zinc contents of the ryegrass crop were low.

KEYWORDS: Bentonite; winery sludge; soil amendment; nutrients

INTRODUCTION

Farming and the food processing industry generate wastes that must be disposed of in ways that are environmentally harmless and, if possible, beneficial. Winemaking is an important agroindustry in a growing number of countries, and one of its significant waste products is bentonite fining sludge: bentonite that has been removed from wine following its addition to adsorb proteins and other causes of haze. The use of bentonite for wine fining has been a common practice for the past 60 years or more (1); its efficacy derives in part from the acid pH of wine and must, in which proteins with isoelectric points of about 5.8–8.0 acquire positive charge and are attracted to the negatively charged bentonite, although steric factors prevent adsorption of proteins heavier than about 45 kDa (2). Given the typical bentonite dosage range of 80–120 g hL⁻¹ (3) and an annual national wine production of about 4 GL (4), the bentonite throughput of Spanish wineries is about 4000 tonnes a year, and their annual bentonite sludge production is this figure plus the weight of adsorbed proteins and other impurities. This estimate gives some idea of the size of the bentonite waste disposal problem.

Because bentonite is a clay and the adsorbates it acquires during wine fining are of plant origin, it is reasonable to suppose that its dispersion in the soil at moderate dosage levels would not be environmentally harmful. Indeed, because its major adsorbate during wine fining is protein, it may act as a fertilizer. However, there are few empirical data in this regard. When Croker et al. (5) studied the properties and fertility of sandy tropical soils treated with bentonite used for vegetable oil fining, they found that crop yield was increased by dosages of up to 20 g kg⁻¹ but that cation exchange capacity (CEC) was hardly altered by a dose as high as 40 g kg⁻¹, presumably because of its oil content. Saviozzi et al. (6) reported that winery sludge obtained through aerobic depuration of wastewaters from a winery, at application rates of 0.5–2.5%, increased soil fertility and microbial activity. It must also be kept in mind that bentonite used for fining wine produced from grapes treated with traditional copper-based fungicides may have acquired a significant copper content. Prior to the disposal of bentonite sludge of a given origin (winemaking, oil fining, etc.) on agricultural soil of a given type, it is desirable to establish its influence on the properties and fertility of the soil in question and to establish dosage levels that optimize cost/benefit ratios while ensuring nontoxicity.

In this greenhouse study, waste bentonite from a local winery was applied to a low-yielding acid soil of Galicia (northwest Spain), and its effects on soil properties and on the growth of *Lolium multiflorum* were evaluated, with particular attention to

* Corresponding author: tel., +34988387000; fax, +34988387001; e-mail, jsimal@uvigo.es.

[†] Soil and Agricultural Science Group, Plant Biology and Soil Science Department.

[‡] Chemical Engineering Department.

[§] Nutrition and Bromatology Group, Analytical and Food Chemistry Department, Faculty of Food Science and Technology.

Table 1. Characteristics of Bentonite before (B) and after (WB) Being Used for Wine Fining^a

parameter	unit	B	WB
pH _(H₂O)		10.1	4.5
pH _(KCl)		10.1	4.7
C	g kg ⁻¹	3.1	294.0
N	g kg ⁻¹	<0.5	28
protein	g kg ⁻¹	<0.1	164
tartrate	g kg ⁻¹	<1	48
electrical conductivity	μS cm ⁻¹	567	5100
P _{Bray}	mg kg ⁻¹	61	584
Na _e	cmol ₍₊₎ kg ⁻¹	33.6	0.8
K _e	cmol ₍₊₎ kg ⁻¹	0.8	118.0
Ca _e	cmol ₍₊₎ kg ⁻¹	7.5	3.4
Mg _e	cmol ₍₊₎ kg ⁻¹	1.9	1.5
Al _e	cmol ₍₊₎ kg ⁻¹	0.1	0.1
Na _t	g kg ⁻¹	17.0	2.0
K _t	g kg ⁻¹	3.9	107.9
Ca _t	g kg ⁻¹	16.5	2.3
Mg _t	g kg ⁻¹	13.0	3.5
Cu _t	mg kg ⁻¹	20	1481
Zn _t	mg kg ⁻¹	147	37
Mn _t	mg kg ⁻¹	87	37

^a e, exchangeable; t, total.**Table 2.** Main Physical and Chemical Characteristics of the Soil Used^a

property	unit	
sand	%	63
silt	%	26
clay	%	11
organic carbon	%	0.5
pH _(H₂O)		4.8
pH _(KCl)		3.8
Na _e	cmol ₍₊₎ kg ⁻¹	0.24
K _e	cmol ₍₊₎ kg ⁻¹	0.03
Ca _e	cmol ₍₊₎ kg ⁻¹	0.30
Mg _e	cmol ₍₊₎ kg ⁻¹	0.27
Al _e	cmol ₍₊₎ kg ⁻¹	0.80
CEC	cmol ₍₊₎ kg ⁻¹	1.64
P _{Bray}	mg kg ⁻¹	75
Cu _t	mg kg ⁻¹	21
Mn _t	mg kg ⁻¹	65
Zn _t	mg kg ⁻¹	70

^a e, exchangeable; t, total.

nutrient content and to heavy metals that might prevent use as fertilizer or soil amender.

MATERIALS AND METHODS

Bentonite and Waste Bentonite. Bentonite (B; 100% granulated montmorillonite, from Laffort) was used without any prior treatment to fine a white wine in a local winery. The resulting sludge (waste bentonite, WB) was dried in air and passed through a 1 mm mesh sieve. Bentonite and waste bentonite properties were determined following the procedures described below for soil samples. Protein was determined as per Lowry et al. (7) following extraction for 24 h with 0.5 M NaOH at 30 °C. **Table 1** show the comparison between the results of both materials. The presence of tartrate species in waste bentonite was measured as tartaric acid after acid extraction with 6 N HCl for 12 h at 20 °C (solid/liquid ratio 1:150 g/mL). Tartaric acid quantification was done by HPLC using an ion-exclusion column for organic acids separations (Transgenomic, Inc., Omaha, NE).

Soil Treatments and Pot Preparation. Table 2 lists the salient physical and chemical properties of the soil used, the C horizon of an Arenic regosol (8) that had developed from a two-mica granite. After drying in air and passage through a 2 mm mesh sieve, it was mixed with waste bentonite in proportions of 1, 2, 5, 10, 20, and 30 g of waste bentonite per kg of soil. Control soil contained no waste bentonite.

PVC pots 12 cm high and 13 cm in diameter were filled to a depth of 2 cm with 0.5–1.0 cm gravel and then made up to about three-fourths of their volume with control soil or a soil-and-waste mixture. Four pots were used per treatment, making 28 in all.

Crop Growth. On day 0 each pot was sown with 150 seeds of the fast-growing species *L. multiflorum* (Italian ryegrass) and was then left in a greenhouse with day and night temperatures of 25 ± 3 °C and 15 ± 3 °C, respectively; soil moisture was maintained at 15% by addition of distilled water every 12 h to recover the initial weight of the pot. The number of seeds that had germinated was counted on days 3 and 10. The plants were removed from the pots on day 35, the aerial part of each was separated from the root, and each part was washed successively with distilled water and 0.001 M HCl before being placed for 3 min in an ultrasound bath to remove any remaining substrate. The plant material was then washed again with distilled water, dried to constant weight in an oven at 65 °C, and weighed. Once the plants had been removed, substrate samples were taken from each pot for analysis.

Laboratory Germination Experiments. A 1:10 (w/v) suspension of bentonite waste in distilled water was shaken for 1 h and filtered. The bottom of each of six Petri dishes was lined with filter paper, and the paper was wetted with the bentonite filtrate in three and with distilled water in the other three. Fifty *L. multiflorum* seeds were placed in each dish, and the percentage that had germinated was recorded on days 3, 7, and 10.

Soil Analyses following Plant Collection. General Details. Soil pH was measured in a 1:2.5 soil/solution suspension in H₂O or 0.1 M KCl using a pH-meter with a combined glass electrode. Total nitrogen and organic carbon were determined in a ThermoFinnigan 1112 Series NC elemental analyzer. Sand (0.05–2.00 mm), silt (0.002–0.050 mm), and clay (<0.002 mm) were determined by the pipette method following sieving (9). Electrical conductivity was measured in filtered extracts obtained from 1:10 soil/water suspensions. Exchangeable cations were extracted with 0.2 M NH₄Cl (10) and were measured by atomic absorption (Ca_e, Mg_e) or flame emission (Na_e, K_e) spectrophotometry. Exchangeable Al (Al_e) was determined by displacement with K in 1 M KCl and subsequent determination of Al by atomic absorption spectrophotometry. CEC was calculated as the sum of Ca_e, Mg_e, Na_e, K_e, and Al_e contents (11). Nitrate nitrogen (N–NO₃) and ammonium nitrogen (N–NH₄⁺) were determined by steam distillation with MgO and Devarda's alloy. Available phosphorus was determined by the Bray II assay (12).

Selective Extractions. Copper, zinc, and manganese were determined in extracts obtained with the extractants below (subscripts used to identify the results of these determinations are indicated in parentheses). In all cases, metals were determined in the extracts by atomic absorption spectrophotometry following centrifugation for 15 min at 4000 rpm and filtration of the supernatant. For quality assurance and control (QA/QC), three certified reference materials (CRM 141 calcareous loam soil, CRM 143 sewage sludge amended soil, and SMR 1570a spinach leaves) were selected. Samples of reference standard material were digested in triplicate and analyzed using the below-mentioned methods. Satisfactory recoveries of Cu, Zn, and Mn in standard reference materials were found showing values that in all cases were within the certified values.

Distilled Water (w). A soil sample was shaken with distilled water in a 1:20 soil/solution ratio. This was based in the method proposed by Ma and Uren (13).

1 M Sodium Nitrate (e). The same procedure was used as for distilled water but using a 1:4 soil/solution ratio. Metals in this extraction are assumed as occupy exchangeable sites in soil colloids (14).

Ethylendiaminetetraacetic Acid (EDTA; ED). A 5 g soil sample in 50 mL of a 0.5 M NH₄OAc solution of pH 4.65 containing 0.02 M EDTA was shaken for 1 h (15). Metals extracted with EDTA were considered as available or potentially available for uptake by plants.

Sodium Pyrophosphate (p). A 1 g soil sample in 100 mL of 0.1 M sodium pyrophosphate (pH 10.1) was shaken for 16 h (16). This extractant is assumed to recover organically bound metals.

Oxalic Acid–Ammonium Oxalate Buffer (o). A 1 g soil sample was shaken for 4 h in 50 mL of 0.2 M buffer (pH 3) (17). The oxalate

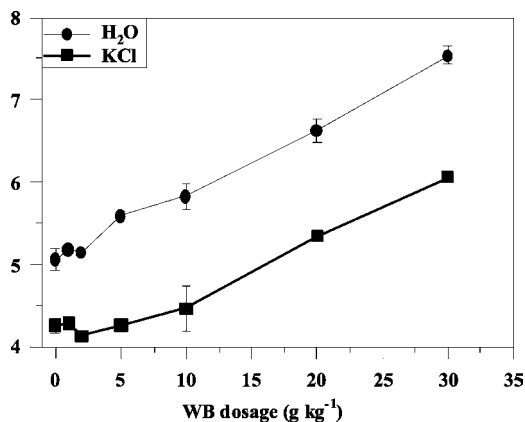


Figure 1. Effects of waste bentonite (WB) on soil pH in water and KCl.

reagent extracts organically bound metals plus those associated to noncrystalline oxyhydroxides of Al and Fe.

Oxalic Acid–Ammonium Oxalate–Ascorbic Acid Buffer (oa). A 1 g soil sample was shaken for 30 min in 50 mL of 0.1 M buffer at 96 °C (18). This treatment is thought to extract the same components as the oxalate extraction but also metals bounded to crystalline oxyhydroxides of Fe.

Nitric Acid–Hydrofluoric Acid–Hydrochloric Acid (t). A 0.5 g soil sample in a mixture of 5 mL of HNO₃, 4 mL of HF, and 1 mL of HCl was microwaved for 30 min at 100 psi to solubilize total metal contents.

Plant Analyses. Once dried, the aerial parts and roots were finely ground, transferred to Pyrex tubes, and treated for 72 h with 65% HNO₃ at 80 °C (19). Cu, Zn, and Mn were determined in the filtered digestate by atomic absorption spectrophotometry.

Statistical Analyses. This was done with the Statgraphics statistical software. Analysis of variance was performed at the 95% probability level to determine the existence of significant differences between groups. If there were significant differences among group averages, the multiple range tests were performed by constructing intervals for the pairwise differences to see between which pair of groups the differences on average are significant at the 95% confidence level. Groups detected as different were marked with different letters (a, b, c, etc.), while groups detected as identical were marked with the same letter. Basic and descriptive statistics together with linear regressions were also performed.

RESULTS

Fining-Induced Changes in the Characteristics of Bentonite. Wine fining reduced the pH of bentonite from 10.1 to 4.5 while increasing carbon content from 3.1 to 294.0 g kg⁻¹, nitrogen from <0.5 to 28.0 g kg⁻¹, Bray II available phosphorus from 61 to 584 mg kg⁻¹, and protein from <0.1 to 164 g kg⁻¹ (Table 1). Electrical conductivity increased from 567 to 5100 μS cm⁻¹. Exchangeable K content increased from 0.8 to 118.0 cmol₍₊₎ kg⁻¹, while exchangeable Na, Ca, and Mg all fell; total CEC increased from 43.9 to 123.8 cmol₍₊₎ kg⁻¹. Total K content increased from 3.9 to 107.9 g kg⁻¹, and total Cu content increased from 20 to 1481 g kg⁻¹; however, total Na, Ca, Mg, Zn, and Mn contents all decreased (probably due simply to dilution with the adsorbed elements). Tartaric acid content in waste bentonite achieved values of 48 g kg⁻¹ (Table 1).

Soil Characteristics after Plant Growth. Although the waste bentonite itself had a pH of less than 5 in both water and KCl (Table 1), the pH of bentonite-treated soil, as measured after collection of the ryegrass crop, rose with added waste bentonite at the rate of about 1 pH unit per 12 g kg⁻¹, values higher than pH 7 being attained with the largest bentonite dosages (Figure 1). Soil available phosphorus content was hardly affected by addition of bentonite except at the highest dosage, which increased P_{Bray} from about 75 to 90 mgkg⁻¹ (Figure 2).

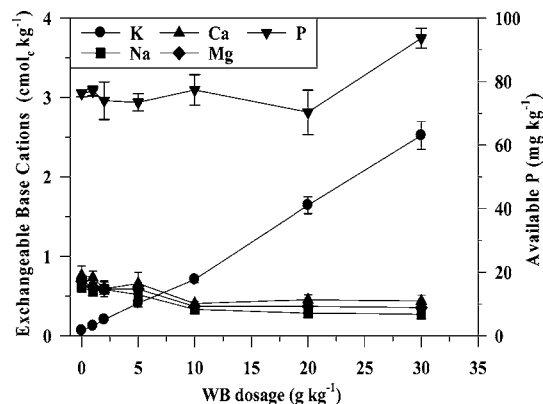


Figure 2. Effects of waste bentonite (WB) on soil exchangeable cation and available (Bray II) phosphorus contents.

Exchangeable Na, Ca, and Mg contents were also unaffected, but K_e content rose with increasing bentonite dosage from 0.1 to 2.5 cmol₍₊₎ kg⁻¹ (Figure 2). N–NH₄⁺ content varied between 0.02 and 0.08 mg kg⁻¹, and N–NO₃ content was always below 0.001 mg kg⁻¹.

Total manganese content remained more or less constant as bentonite dosage increased, as did Mn_{ao} (Figure 3a). Contents of other Mn extractions (Mn_w, Mn_e, Mn_p, and Mn_o) were always less than 4 mg kg⁻¹; the largest fraction was always residual Mn (Mn_t – Mn_{ao}). Zinc behaved similarly (Figure 3b). Total copper content was always less than zinc or manganese content but rose steadily with increasing bentonite dosage as a result of the high copper content of the waste bentonite (Table 1). The levels of Cu_{ED}, Cu_p, Cu_o, and Cu_{ao} also rose with bentonite dosage (Figure 3c), but those of Cu_w or Cu_e did not, which both remained less than 2 mg kg⁻¹. Although the waste bentonite had a Cu_w content of 443 mg kg⁻¹ (almost 30% of its total Cu content), in the soil this soluble Cu was bound to organic matter (Cu_p), which for dosages greater than 2 g kg⁻¹ increased progressively with dosage to make up more than 30% of soil copper and give rise to a corresponding reduction in the relative size of the residual copper fraction.

Germination. For waste bentonite dosages up to 10 g kg⁻¹, about 20% of pot-sown seeds had germinated by day 3 and about 70% by day 10 (Table 3). At higher dosages, germination rates were lower, about 2% by day 3 and about 45% by day 10. In the laboratory experiment, germination was significantly inhibited by the waste bentonite extract (Figure 4).

Crop Yield. Aerial and root ryegrass biomass on day 35 increased with waste bentonite dosage up to a dosage of 5 g kg⁻¹ and fell with increasing dosage thereafter (Figure 5). Manganese content was higher in aerial parts than in the roots, and at low bentonite dosages aerial Mn content fell while root Mn content rose; however, in neither the aerial parts nor the roots did the Mn content ever exceed 0.8 mg kg⁻¹ (Figure 6a). Zinc concentrations ranged from about 1.5 to about 3.0 mg kg⁻¹, without significant differences between the aerial parts and the roots (Figure 6b). Copper contents were higher in the roots than in the aerial parts, especially for bentonite dosages of 10 g kg⁻¹ or more, but a concentration of 0.4 mg kg⁻¹ was exceeded only in the roots of plants grown with the highest bentonite dosage (Figure 6c).

DISCUSSION

Apart from lowering its pH, the use of bentonite for wine fining increased its C, N, P, and K contents, thus endowing it with considerable potential as a fertilizer. As a source of nitrogen

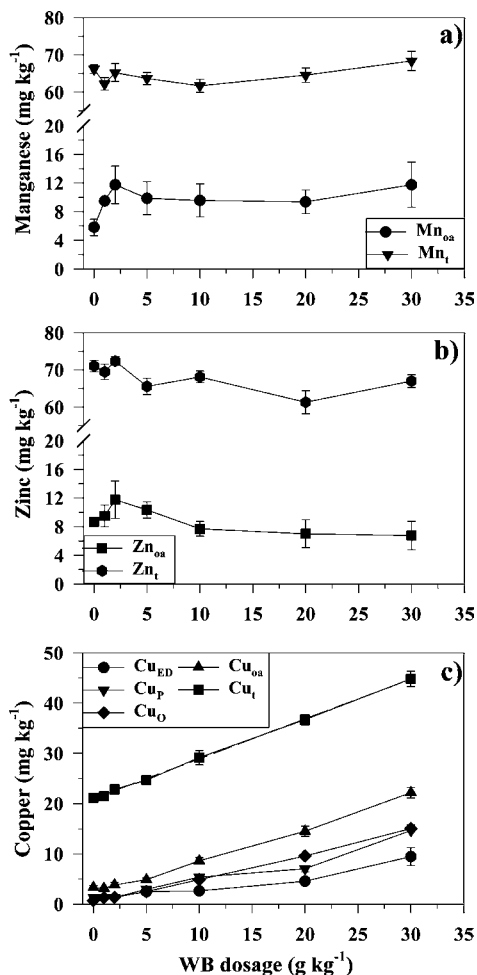


Figure 3. Effects of waste bentonite (WB) on total soil Mn (a), Zn (b), and Cu (c) contents and the fractions extractable with certain extractants: EDTA (ED), sodium pyrophosphate (p), oxalic acid–ammonium oxalate buffer (o), oxalic acid–ammonium oxalate–ascorbic acid buffer (oa), and nitric acid–hydrofluoric acid–hydrochloric acid (t, total).

Table 3. Germination of *L. multiflorum* Seeds in the Greenhouse Experiment, for each Dosage of Waste Bentonite (WB)^a

time (days)	WB dosage (g kg ⁻¹)						
	0	1	2	5	10	20	30
3	19 ± 2 ^a	18 ± 1 ^a	16 ± 3 ^a	18 ± 1 ^a	21 ± 2 ^a	1 ± 1 ^b	3 ± 1 ^b
10	72 ± 8 ^a	66 ± 5 ^a	71 ± 4 ^a	69 ± 2 ^a	77 ± 6 ^a	40 ± 1 ^b	51 ± 4 ^b

^a Mean percentages ± standard deviations; *n* = 4. Time groups detected as different were marked with different letters (a, b, c, etc.).

the used bentonite is likely to act in slow-release fashion, because a large part of its 28 g kg⁻¹ N content belongs to protein and will undergo successive processes of ammonification and nitrification. Its P content, 584 mg kg⁻¹, is of particular relevance for acid soils such as that used in this study, which are often P-deficient because of the immobilization of phosphorus by Fe and Al oxides (20, 21). Some 43% of its 108 g kg⁻¹ K content is exchangeable (NH₄Cl-extractable).

The finding that waste bentonite raised soil pH is of economic interest, because it would allow savings in lime-based pH amendments, and is also theoretically intriguing, because the pH of the waste bentonite is less than 5. Although a slight increase in pH would be caused by ammonium hydroxide

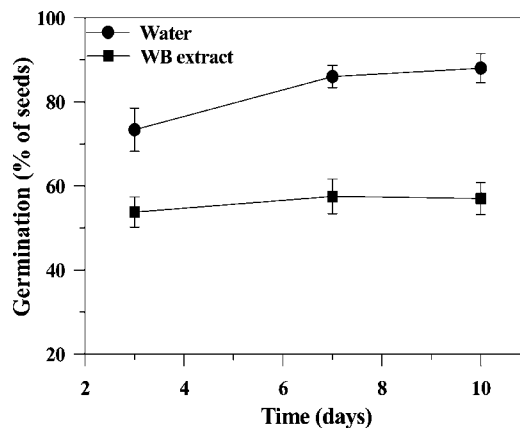


Figure 4. Germination rates of *L. multiflorum* seeds on filter paper impregnated with water and waste bentonite extract.

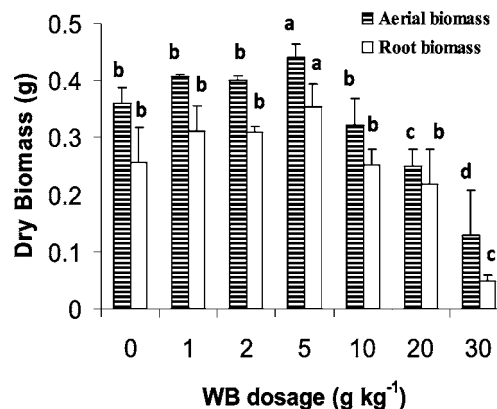


Figure 5. Effects of waste bentonite treatment on the aerial and root biomass of *L. multiflorum* plants.

derived from microbial degradation of the high protein content of the waste bentonite, such an increase could hardly be as large as was observed (2 pH units at the highest bentonite dosage). It seems possible that at the pH of the soil (pH 5), potassium tartrate formed during the drying of the waste bentonite may have undergone conversion to potassium bitartrate, removing protons from the soil solution and releasing potassium (part of which would be taken up by plants and thereby increase bitartrate formation by raising pH). This hypothesis is supported by the fact that the K_e content of treated soil is greater than the sum of the K_e contents of waste bentonite and untreated soil, especially at the higher dosage levels, in spite of its partial removal by plants (Table 4). Moreover, tartrate content measured as tartaric acid in waste bentonite showed a significant increase related to its value in purchased bentonite (Table 1).

Treatment with waste bentonite raised soil CEC from 1.6 cmol(+) kg⁻¹ to 3.6 cmol(+) kg⁻¹ at the highest bentonite dosage and altered the composition of the exchange complex, which was dominated by Al in the untreated soil and by K in waste bentonite. Increased CEC is of interest both for fertility and for environmental protection, because it increases the capacity of the soil to retain potential pollutants and prevent their reaching water bodies. The fertility of tropical soils has been reported to benefit from treatment with both bentonite used for vegetable oil fining (5) and beneficiated bentonite (22), and in the latter case CEC also rose.

The aerial and root biomass of the ryegrass crop increased with waste bentonite dosage up to a dosage of 5 g kg⁻¹ but is reduced at higher waste bentonite dosages. This nonlinear

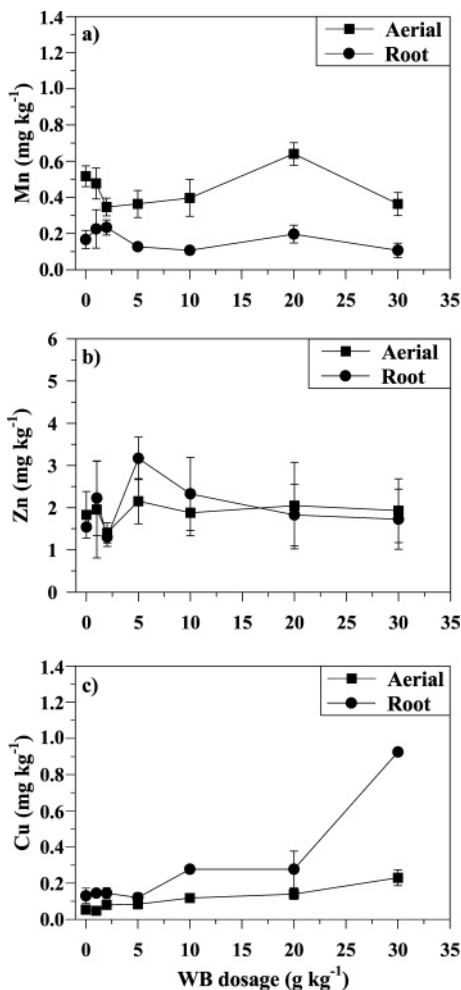


Figure 6. Effects of waste bentonite (WB) dosage on the Mn (a), Zn (b), and Cu (c) contents of the aerial parts and roots of *L. multiflorum* plants grown on WB-treated soil.

Table 4. Exchangeable Potassium ($\text{cmol}_{(+)} \text{kg}^{-1}$), as Determined by Extraction with Ammonium Chloride ($K_{\text{e-D}}$) and as Calculated from the Contents of the Untreated Soil and the Waste Bentonite ($K_{\text{e-R}}$), for each Waste Bentonite (WB) Dosage^a

	WB dosage (g kg^{-1})						
	0	1	2	5	10	20	30
$K_{\text{e-R}}$	0	0.06	0.12	0.295	0.59	1.18	1.77
$K_{\text{e-D}}$	0.07	0.012	0.20	0.41	0.71	1.64	2.52

^a Slope of $K_{\text{e-R}}$ vs $K_{\text{e-D}} = 0.713 \pm 0.037$ (coefficient estimate \pm 95% confidence interval).

dependence of biomass on bentonite dosage may possibly derive from the following two main causes.

(a) For most crops the optimal soil K/Mg ratio is 0.2–0.3 (23). As the waste bentonite dosage increased in this study, the K/Mg ratio of the K-deficient untreated soil (0.1) was first corrected but then became indicative of Mg deficiency, ratios of 0.2, 0.3, 0.7, 1.9, 4.4, and 6.5 being attained with dosages of 1, 2, 5, 10, 20, and 30 g kg^{-1} , respectively.

(b) Water extract of waste bentonite has an electrical conductivity of 5100 $\mu\text{S cm}^{-1}$ and high concentrations of phosphates (179 mg L^{-1}) and sulfates (1414 mg L^{-1}). This results in a high osmotic potential that subjects the plants to water stress. The high electrical conductivity of winery-derived wastes was also observed by Saviozzi et al. (6).

The beneficial effects of soil amendments must not be accompanied by undesirable side effects such as increased heavy metal levels. For agricultural use of sewage sludges, for example, Council Directive 86/278/EEC (24) limits copper and zinc input to 12 and 30 $\text{kg ha}^{-1} \text{ year}^{-1}$, respectively (no limit is set for manganese input). Application of the waste bentonite used in this study at a rate of 5 $\text{g kg}^{-1} \text{ year}^{-1}$ would result in copper and zinc inputs well within the limits established for sewage sludge. Furthermore, the major heavy metal in this waste is copper, and the phytotoxicity of this content is reduced by its soluble fraction being immobilized (mainly in organic colloids) when applied to the soil, as was corroborated by the finding that plant copper content was similar to Mn content and less than Zn content. Thus in spite of the high copper content of the waste bentonite, it seems unlikely that the use of this waste as a fertilizer and soil amendment would introduce large quantities of copper into the food chain.

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