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Agriculture and Environment

Original article

Capacity of soil amendments in lowering the phytoavailability of sludge-borne zinc

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Abstract – One way to reduce the phytoavailability of an excess of metals such as zinc in soil is through the addition of amendments. The effectiveness of inorganic materials such as basic slags, magnetite, maghemite, hematite, birnessite, hydrous manganese oxide, steel shots, and beringite, was evaluated in a pot experiment with a coarse sandy soil contaminated by sludge-borne zinc. Zinc extractability and phytoavailability were investigated using single soil extraction by 0.1 M calcium nitrate and vegetation experiments with dwarf bean (*Phaseolus vulgaris* L.) and ryegrass (*Lolium multiflorum*). A phytotoxicity test based on the activities of enzymes involved in the stress metabolism caused by toxic Zn concentrations in the primary leaves of dwarf beans was also performed. Based on the addition rate, birnessite (10 g·kg^{-1} soil, dry weight DW) resulted in the maximum decrease in extractable Zn from the contaminated soil. Beringite gave similar results but at 50 g·kg^{-1} soil DW. Birnessite and beringite treatments were the most effective to reduce Zn assimilation by dwarf bean, and in consequence phytotoxicity. Subsequent harvests of ryegrass confirmed the beringite effect on Zn uptake 5 months following the soil treatment. For birnessite, Zn availability to ryegrass shoots increased however at the third harvest and reached the level of the untreated Zn-contaminated soil.

beringite / iron oxide / manganese oxide / soil remediation / zinc

Résumé – Capacité d'amendements à diminuer la phytodisponibilité du zinc issu de boues urbaines dans un sol. Amender le sol est une des voies possibles pour diminuer l'exposition excessive des racines des végétaux aux métaux comme le zinc. L'efficacité de matériaux minéraux, e.g. scories phosphatées, magnétite, maghémite, hématite, birnessite, oxyde de manganèse hydraté, grenaille d'acier et béringite, pour diminuer la phytodisponibilité de Zn a été étudiée en vase de végétation avec un sol sableux contaminé par des apports de boues urbaines. L'extractabilité et la phytodisponibilité de Zn ont été déterminées à l'aide d'une extraction sélective au nitrate de calcium 0,1 M et de cultures de végétaux, haricot nain (*Phaseolus vulgaris* L.) et raygrass (*Lolium multiflorum*). Un test de phytotoxicité, basé sur les

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Communicated by Marco Trevisan (Perugia, Italy)

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activités d'enzymes permettant à la plante de compenser des effets négatifs du zinc, a été réalisé avec les feuilles primaires du haricot. D'après le taux d'apport, la birnessite (10 g·kg⁻¹ sol sec) a diminué le plus la quantité de Zn extractible du sol. La béringite produit un effet similaire, mais avec un taux de 50 g·kg⁻¹ sol sec. Birnessite et béringite sont les traitements les plus efficaces pour diminuer le prélèvement du zinc par le haricot et la phytotoxicité. Trois coupes successives du raygrass ont montré que la diminution du prélèvement de Zn par la plante après traitement du sol par la béringite persiste sur 5 mois. Avec la birnessite, l'accumulation de Zn dans les parties aériennes du raygrass augmente à la troisième coupe, redevenant similaire à celle des plantes cultivées sur le sol non traité.

beringite / oxyde de fer / oxyde de manganèse / remédiation du sol / zinc

1. Introduction

For many years, Zn has ranked fourth in annual world metal consumption [1, 4]. This often results in excessive build-up of anthropogenic Zn in topsoils caused by various activities such as industry without efficient emissions' controls [5, 25, 34], long-term use of organic wastes (sewage sludge, slurry) [2, 11, 19], and of sediments dredged from ship canals and rivers [1]. Increase of Zn in soil may create an environmental problem due to its persistence in the environment, mobility, bioaccumulation and/or phytotoxicity. Consequently, a report on soil quality by the Commission of the European Community focused attention on Zn as a soil contaminant [14]. In higher plants, the visual symptoms of Zn-toxicity are often chlorosis and stunted growth, and inhibition of root growth is usually manifested [10]. Critical concentrations in leaves above which the yield decreases are often at 200 mg·kg⁻¹ dry matter (DM) [22, 31]. Toxic Zn concentrations inhibit physiological processes such as photosynthesis, phloem translocation, and transpiration [13, 31]. Soil reclamation reducing Zn availability is required in two situations: (i) for safer food production and maintenance of crop yield in slightly polluted soils; and (ii) for the reclamation of highly-polluted soils that are often denuded of vegetation. Both ecological and engineering technologies have been developed for treating metal-contaminated soils [12, 20, 25, 27, 35]. Metal in situ immobilisation may be a more realistic and cost-effective alternative, especially for vast industrial sites and surrounding rural land, dumping grounds, and sludge-treated soils highly contaminated due to historical pollution [35].

Both physico-chemical and biological soil factors control Zn bioavailability. Therefore, a logical solution for managing Zn-polluted soil, and especially to minimise plant uptake, involves a two-way approach [9]. First, plant species differ in both metal uptake and sensitivity to metals. Monocotyledonous crops such as corn and most grasses can generally tolerate higher soil Zn concentrations compared to dicotyledonous plants. Differences among cultivars in Zn uptake have also been described [10, 18]. On the other hand, soil properties controlling Zn phytoavailability are: soil pH, organic matter, cation exchange capacity (CEC), clay type and amount, oxides and redox potential [1, 2]. Amelioration of Zn-contaminated soils is usually based on the application of lime and/or organic matter [8, 12, 20]. In most cases, but especially in acidic sandy soils, lime and phosphate (phospho-gypsum, basic slag) are effective in lowering Zn uptake by plants. However, the sustainability of the liming effect is generally short or its effect is less than expected [27, 35]. Excessive increases in pH may enhance metal complexing by soluble organic matter, desorption of toxic oxyanions in mixed metal systems, and nutrient deficiency. Candidates for inorganic amendments are oxides, silicates and phosphates. Several materials have been shown to decrease either Zn mobility or Zn availability after a single application. Beringite, a cyclonic ash provided by the fluidized bed burning of coal refuse from the former coal mine in Beringen, Belgium, can restore plant growth by both reducing Zn exposure at the root level and increasing Ca and Mg nutrition [27, 33, 34]. Zinc sorption on hydroxyapatite surface has been reported [37]. Fe-bearing material such as steel shots, hydrous Mn oxides, apatite and zeolites have been

found to reduce Zn uptake by plants in mesocosms [12, 17, 25, 27, 28]. A comparison of the Zn immobilising effect of hydrous oxides and alkaline materials was unavailable from a literature review, except for a study on Fe-rich[TM], lime and apatites [12, 27]. Few information is available on the relative effectiveness of soil amendments in decreasing the phytoavailability of sludge-borne Zn. Therefore, this study is aimed at comparing the capacity of Fe and Mn oxides, and of three alkaline materials for the abiotic remediation of a soil contaminated with sludge borne Zn.

Zinc has accumulated in excess in a coarse sandy soil due to applications of sewage sludge at the INRA Couhins experimental farm, Bordeaux, France [19]. As a result, Zn phytotoxicity occurred on sensitive plant species such as dwarf bean. A immobilising treatment was developed at laboratory scale to render sludge-borne Zn less mobile in this soil and to reduce Zn uptake by plants. Amendments tested on a laboratory scale were iron oxides, manganese oxides, cyclonic ashes (beringite), basic slags and lime. Zn extractability and phytoavailability were investigated using single soil extraction by 0.1 M calcium nitrate and vegetation experiments with dwarf bean and ryegrass. The activities of enzymes such as peroxidases and several NADPH producing enzymes of the intermediary metabolism were determined in the primary leaves of dwarf bean to test the beneficial effect of amendments on plant metabolism.

2. Materials and methods

2.1. Soils and amendments

Soils were sampled in the 0 to 20 cm top layer of two plots located in a long term field trial with sewage sludge applications on an Arenic Udifluvent soil at the INRA Couhins experimental farm, Bordeaux, France. This soil was developed on colluvial material, and was initially acidic (pH$_{water}$ = 5.5). The experiment has been described previously [19]. High Zn content had built up in the contaminated plot due to the application of

Table I. Main characteristics of the control (AMB0) and Zn-contaminated (AMB100) soils.

	AMB0	AMB100
Sand (g·kg⁻¹)	867	811
Silt (g·kg⁻¹)	88	126
Clay (g·kg⁻¹)	45	63
Organic matter (g·kg⁻¹)	21	42
C/N	11.2	9.1
pH (water)	5.4	5.8
C.E.C. (Metson) (cmol·kg⁻¹ soil DW)	3.3	9.4
Soil metals (mg·kg⁻¹ soil DW) (aqua regia extract)		
Cd	0.3	5.7
Cu	14	67
Cr	6.8	23
Fe	2807	9313
Mn	33	1797
Ni	2.4	31
Pb	18	189
Zn	19	1074

100 mg sludge DW·ha⁻¹ every 2 years since 1974 (Tab. I). The main characteristics of the Zn-contaminated soil (so-called untreated soil below, and abbreviated AMB100) and the control soil (AMB0) are listed in Table I.

Nine materials were used to treat the AMB100 soil. Such amendments may induce changes in soil pH and/or enhance adsorption [27]. Thomas phosphate basic slags (TBS) are a steel metallurgy byproduct (SNST, Puteaux, France). This material contains phosphoric acid (6.6% P_2O_5), lime (32% CaO), Mg (10% MgO), Si (4.7%) combined as calcium silicophosphates, Al (10.3%), Mn (1.6%), Fe (13.4%) and Cu (166 mg·kg⁻¹ DW) [28]. Beringite (BER) is a modified aluminosilicate that originates from the fluidized bed burning of coal refuse (mine pile material) from the former coal mine in Beringen, Belgium [27, 34]. Its pH is strongly alkaline (~ 11) as it contains CaO and MgO [35]. SiO_2 and Al_2O_3 represent 52% and 30% of the whole product. Illite is the dominant clay present; during the schists heating, kaolinite, chlorite, and

pyrite disappeared whereas ettringite was formed. Iron oxides includes maghemite (γFe_2O_3, MAGH), magnetite (Fe_3O_4, MAGN), and hematite (αFe_2O_3, HEM) supplied by Magnox Pulaski Inc, and a Fe-bearing material, steel shot (SS) which contains mainly αFe and some impurities [22]. Manganese oxides were a Na-birnessite (($Na_{0.30}$ $Mn^{II}_{0.05}$) inter-layer ($Mn^{IV}_{0.74}$ $Mn^{III}_{0.21}$ □ $_{0.05}$) layer $O_{\sim 2}$), (BIRN), and a hydrous manganese oxide (HMO). Na-bir-nessite was prepared at pH 9-10 by oxidising $Mn(OH)_2$ [16]. HMO was synthesised by reducing potassium permanganate in acidic conditions [15]. Calcium oxide (CaO, Prolabo) with particle size < 0.3 mm was used in combination with HMO. Seven amendments were given separately in the contaminated soil, whereas CaO was combined with HMO to neutralise its acidity. Application rates of each amendment except CaO were based on preliminary bench-scale experiments [27]. The same rate, 10 g·kg^{-1} soil DW, was used for HEM, MAGN, MAGH, BIRN and SS. But BER is ineffi-cient at this rate, and was added at 50 g·kg^{-1} soil DW. Immobilisation of metals such as Cd in soil following SS and HMO addition is enhanced at high soil pH [28]. Therefore, alkaline material such as TBS (2 g·kg^{-1}) was used alone and in combina-tion with SS (10 g·kg^{-1}). HMO (10 g·kg^{-1}) was combined with CaO (0.5 g·kg^{-1}). Rates of TBS and CaO were determined at the bench scale prior to this experiment. Amendments or combinations (e.g. HMOCaO, SSTBS) were separately mixed with AMB100 air-dried soil, previously sieved to 4 mm, by rotating the plastic flask containers. Soil treatments were replicated four times. For each treatment, three pots were cultivated and a fourth one was used for soil extractions. The soil DW inside each pot was 1.2 kg. All potted soils were rehydrated to about 70% field capacity with a stan-dard nutrient solution containing 250 mg N (NH_4NO_3), 207 mg K (K_2SO_4), 60 mg Mg ($MgSO_4$), and 109 mg P ($Ca(H_2PO_4)_2$) per kg soil DW, and placed under greenhouse conditions.

2.2. Plants

After one, month to permit reaction with the soil matrix, dwarf beans (cv. Limburgse vroege) were sown (3 seeds per pot). Seedlings were grown in a growth chamber (16 h light, photosynthetic active radiation set at 350 μmol·m^{-2}·s^{-1}, 25 °C, 75% rela-tive humidity; 8 h darkness, 20 °C). After sowing, the soil water status was maintained at 50% field capacity by adding deionised water. Bean plant organs (i.e. primary leaves, shoots, and roots) were harvested after a two week growing period. Shoot length, fresh and dry weight of the aerial parts, pri-mary leaf area, and chlorophyll content (Minolta SPAD chlorophyllmeter) were determined. Potted soils were maintained at 50% of the field capacity, and fertilised again with half of the initial amount of nutrient solution. After a two week stabilisation period, a hybrid ryegrass (cv. Augusta) was sown in the pots (1 g of seeds per pot). Ryegrass was harvested three times at four week intervals during its growth period. After each harvest, half of the initial amount of nutrient solution was supplied to the pots.

2.3. Soil analysis

Aliquots of potted soils without plant were col-lected. For each treatment, the potted soil was rehomogenised and then broken up to make repli-cates. In judging Zn availability, only the small proportion of total Zn in the soil available to plant is relevant. One way, especially in comparative studies, is to use the water-soluble and exchange-able fractions in soil as an index of the phyto-avail-able fractions [21]. Dilute salt solutions such as $Ca(NO_3)_2$ are useful extractants to estimate Cd and Zn phyto-availability [21]. Therefore, single extractions were used to assess Zn extractable in treated, untreated, and control soils. Every extrac-tion was replicated three times and a quality assur-ance scheme included blank reagents in every batch. All glassware and plastic flasks were washed with 1.4 M nitric acid, rinsed in distilled deionised water, and dried before use. 10 g of soil DW (air-dried, sieved 2 mm) was shaked (run over run) with 0.1 M calcium nitrate (suprapur, Merck) (50 mL) during 2 h at 20 °C. Extracts were filtered through ash-free paper, acidified with 14 M nitric acid (rectapur) (1 mL) to prevent metal adsorption to glassware, and kept at 4 °C until analysis.

2.4. Plant analysis

Plant tissues were dried at 80 °C to constant weight, weighed, and then milled in a planetary grinder with a zirconium oxide covering (Retsch PM4). Aliquots (1 g DW) were then digested overnight in 14 M HNO_3 (5 mL) and H_2O_2 30% by volume (10 mL), and heated at 120 °C for 2 hours [6]. The solutions were filtered through ash-free filter paper and made to 100 mL with distilled water. Metal concentrations were determined by either flame or graphite furnace atomic absorption spectrophotometry (Varian spectrA A20, A300 with deuterium background correction, and A400 with Zeeman effect), depending on the concentration. For either standards or samples, three absorbance measurements gave a relative standard deviation less than ± 2%. Quality control of metal analyses was performed by analysing two certified samples (ryegrass and sea lettuce reference materials BCR 281 and 279 from the Community Bureau of Reference, Commission of the European Communities) and three blanks included in every batch. Metal contents in certified samples were recovered with less than 5% of variation.

For enzyme measurements, bean primary leaves were collected 14 days after sowing. Enzyme analysis was not performed in the roots because Zn is known to exert no measurable (iso)enzymatic effects at this level [32]; samples of 1 g fresh weight were frozen in liquid nitrogen and stored at –80 °C. Material was homogenised with an ice-cooled mortar and pestle in 5 mL of 0.1 M Tris-HCl buffer pH 7.8, containing 1mM dithiothreitol and 1 mM EDTA. The homogenate was squeezed through a nylon mesh and centrifuged at 12 000 g and 4 °C for 10 min. The supernatant was collected, and on this crude extract, the capacity of the following enzymes was measured spectrophotometrically with a Varian Cary 1E [33]: (gaïacol) peroxidase (GPX; E.C.: 1.11.1.7), malic enzyme (ME; E.C.: 1.1.1.40), glucose-6-phosphate dehydrogenase (G-6-PDH; E.C.: 1.1.1.49), glutamate dehydrogenase (GDH; E.C.: 1.4.4.2) and isocitrate dehydrogenase (ICDH; E.C.: 1.1.1.42). The enzyme activity was expressed in mU per mg soluble protein and per g FW. Soluble protein content in leaf extracts was determined using the Bio-Rad protein assay with serum bovine albumin as standard [7].

2.5. Statistical analysis

Datasets for shoot yields, and metal concentrations in plants and soil extracts were analysed using a one-way ANOVA (STAT.ITCF 4.0); within a column or a bargraph, mean values followed by the same letter are not significantly different at the 5% level (Newman-Keuls test).

3. Results and discussion

The contaminated soil AMB100 contains 1074 mg $Zn·kg^{-1}$ DW as compared to an initial value of 19 mg $Zn·kg^{-1}$ DW in the AMB0 soil (Tab. I). For the purpose of comparison, median and maximum Zn concentrations in French sandy soil layers are 17 mg and 34 $mg·kg^{-1}$ soil DW [5].

3.1. Soil Zn extracted by 0.1 M Ca(NO₃)₂

Among soil samples, extractable Zn was a minimum for AMB0 and maximum for AMB100 (Tab. II). In the AMB100 and AMB0 soils, it represents 2.5% and 7.4 % of total Zn content. Metal availability in soil is often characterised by a distribution coefficient Kd, between the solid phase and the solution at equilibrium [3]. For all soil treatments, the Kd_{Zn} value was here calculated by dividing total Zn content in soil ($mg·kg^{-1}$) by Zn concentration in 0.1 M $Ca(NO_3)_2$ extract ($mg·L^{-1}$). High values of Kd indicate high Zn retention by the solid phase through sorption reactions and low availability for plant uptake. Kd_{Zn} values varied by a factor 25 and ranged from 67.4 to 1724 $L·kg^{-1}$ (Fig. 1). Minimum and maximum values were found for control and beringite-treated soils, respectively. Kd_{Zn} value in AMB100 soil was three fold that in AMB0 soil. Despite a higher value for Zn extracted, Zn retention in AMB100 soil is relatively larger than in AMB0 soil because Zn in AMB100 is predominantly bound at lattice

Table II. Extractable Zn[†], soil pH, and cation exchange capacity (C.E.C.) in the control and the Zn-contaminated soils with and without amendments.

Soil treatment	Extractable Zn	Soil pH (water)	C.E.C. Metson	C.E.C. Cobalti-hexammine
	mg·kg^{−1} soil DW		cmol·kg^{−1} soil DW	
Control soil				
AMB0	1.4	5.9	3.8	4.4
Zn-contaminated soil (AMB100)				
BER	3.1	6.9	8.3	10.2
BIRN	4.6	6.3	10.2	9.7
TBS	11.1	6.2	8.5	9.6
SSTBS	8.6	6.6	10.0	9.1
SS	15.3	6.4	9.9	9.3
MAGN	21.8	5.8	8.5	7.9
MAGH	25.7	5.6	8.6	9.3
HEM	19.7	6.1	10.2	10.9
HMOCaO	14.6	5.9	9.1	9.5
Untreated	26.7	5.8	9.4	8.4

[†] $0.1\ M\ Ca(NO_3)_2$

Figure 1. Changes in Kd_{Zn} (0.1 M calcium nitrate extractable-Zn, mg·L^{−1} vs. total Zn content in soil, mg·kg^{−1}) in relation to soil pH (water) as a result of the addition of amendments to a coarse sandy soil contaminated by sludge-borne Zn.

vacancy sites of the phyllomanganate chalcophanite ($ZnMn_3O_7 \cdot 3H_2O$) [23]. This oxide originates from the large Mn concentration in the sludge applied. Its structure presents similarities with birnessite. All soil treatments except maghemite led to a significant increase in Kd_{Zn} (Fig. 1). The amendment nature and changes in soil pH caused Kd_{Zn} changes. However, other parameters such as the concentration ratio between the sorbed element and the ligands, ionic strength, and the presence of competing ions may contribute. For the AMB100 soil, beringite and birnessite treatments by far delivered the most pronounced increases in the Zn distribution coefficient, 8.6 and 5.4 fold respectively as compared with the untreated soil. However, the beringite amount added to the Zn-contaminated soil was 5 times higher than that of birnessite. Thus, birnessite appeared to decrease extractable Zn to the highest extent. Its large sorption capacity results from its chemical and structural properties [29]. The replacement of Mn^{IV} by Mn^{III} and the presence of layer vacancies ($\square$) in the structure create a deficit of positive charges balanced predominantly by interlayer Na. The exchange capaci-
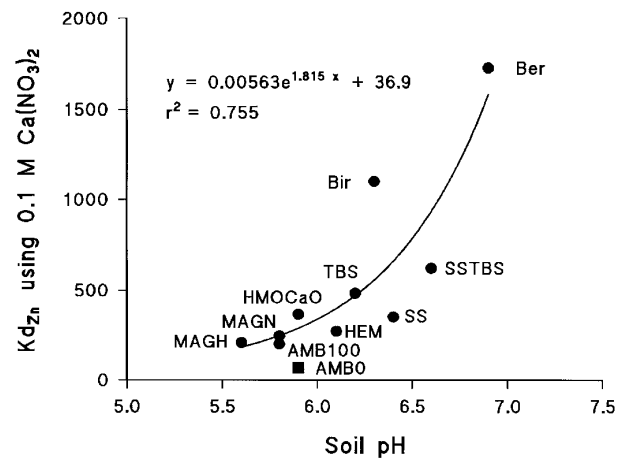
ty of this material is as high as 300 cmol·kg^{−1} [36], three times larger than that of typical montmorillonite. In comparison, the CEC of beringite is equal to 20 cmol·kg^{−1} [27]. When equilibrated in neutral to acidic conditions, Na-birnessite can sorb large metal amounts. Metals form three Me-O-Mn bonds at the birnessite surface [23, 24, 29]. This sorption mechanism accounts for the high binding affinity and low reversibility of metal sorption experiments [23]. Our data confirmed beringite at the 50g·kg^{−1} rate as an efficient amendment for rendering Zn less mobile [33, 34]. Its metal immobilising capacity is supposed to be based on the combination of 3 mechanisms [27, 35]: (i) metals rapidly (hours) adsorbed onto highly accessible sites on the surface of the modified clay and the original soil components freed due to a liming effect; (ii) a slower type (days) of sorption occured such as coprecipitation associated with Al, Fe, and Mn oxides; and (iii) on the longer term (years) minerals such as metal silicates can form and metals diffuse across the mineral surface.

Four other amendments caused a 2.4 to 3 fold increase in Kd_{Zn} when they were applied to AMB100: SS, HMOCaO, TBS, and SSTBS. Mixed together, SS and TBS were more efficient to decrease extractable Zn than when used separately. SS mainly contains α-Fe(98%) but also native Mn

as an impurity (e.g. 7710 mg·kg^{-1} DW) [28]. X-ray diffraction and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy show that native iron was oxidised in this sandy soil to lepidocrocite (γFeOOH), magnetite and maghemite whereas Mn transformed to a birnessite-like phyllomanganate compound [23, 28]. First, the addition of crystallised iron oxides such as hematite, maghemite and magnetite in soil proved unsuccessful for decreasing Zn extracted. Second, Mn content in the shot is only 8 g·kg^{-1} and not all of the steel would oxidise after the one month reaction period. Using X-ray diffraction, it has been reported that some α-Fe is still present in oxidised shots recovered with a magnet from a sandy soil [28]. Thus, reduction in Zn brought about by the addition of steel shots is probably due to the specific surface area of a layer of fresh iron oxides forming on either the shot or soil particles. These in situ newly-formed oxides would have a larger surface area than the bulk oxide minerals added. Newly-formed Mn oxides in SS-treated soils may also enhance Zn inactivation as in the HMO-and birnessite treated soils, but further investigation is needed to quantify their contribution compared with iron oxides. Alkaline compounds such as TBS increased soil pH and the contribution of CaO limited the well-known acidifying effect of HMO (Fig. 1). In the 6.0–6.5 pH range, pH increase can likely enhance Zn sorption on the particles of Fe and Mn-oxides initially present in the contaminated soil, or newly formed following the addition of SS, or directly added as amendments (HMO).

Soil pH was modified following the addition of several amendments into the zinc-contaminated soil (Fig. 1). A relationship was found between soil pH and Kd$_{Zn}$ among data related to AMB100. However, treatments such as MAGN and HMOCaO increased KdZn although soil pH did not vary significantly. Other mechanisms than surface complexation and acid-base reaction may thus be hypothesised. The CEC of soil samples was determined either in a buffered ammonium acetate solution at pH 7 (Metson method) or by respecting soil pH (cobaltihexammine method) (Tab. II). When soil pH was not buffered, an increase in CEC was found following soil treatment, except

with magnetite. This compound had an opposite effect. Soil pH was the highest correlated variable with Kd$_{Zn}$ (r = 0.80, p < 0.01). A weaker correlation (r = 0.44, p < 0.01) was found for the CEC (cobaltihexammine).

3.2. Plant yield

Plant yield could be used as an index of amelioration success. However decrease in yield is not always a proof for metal phytotoxicity. Bean did not germinate when sown in the untreated and limed (0.5 g·kg^{-1}) AMB100 soils (Tab. III). Every other soil treatment enhanced the dry-matter yield of bean plants. Moderate chlorosis was observed on the primary leaves of seedlings cultivated on AMB100 added with either HMOCaO or HEM. Relative to the control treatment, beringite application resulted in an increase in the yield of bean roots. The smallest yield of bean shoots was associated with the HMOCaO addition. Nodules of *Rhizobium* were only found on the roots of plants grown in the control soil.

Table III. Dry-matter yields (g·plant^{-1}, DW) and shoot length (cm) of dwarf beans.

Soil treatment	Shoot length	Shoot yield	Root yield
Control soil			
(AMB0)	9.3 a	1.4 b	0.51 b
Zn-contaminated soil (AMB100)			
Untreated	0	0	0
TBS	8.3 a	1.7 a	0.47 b
SS	7.9 a	1.8 a	0.53 b
SSTBS	8.8 a	2.0 a	0.53 b
MAGN	8.9 a	1.8 a	0.52 b
MAGH	9.1 a	1.9 a	0.61 b
HEM	9.4 a	2.1 a	0.54 b
BER	9.1 a	2.1 a	0.73 a
BIRN	8.6 a	1.7 a	0.59 b
HMOCaO	7.8 a	1.4 b	0.55 b
CaO	0	0	0

Mean values with different letters within a column are significantly different (p < 0.05).

Table IV. Ryegrass shoot yield (g·pot^{-1}, DW).

Treatment	Cut #1	Cut #2	Cut #3
Control soil			
AMB0	2.07 b	1.75 d	2.47 b
Zn-contaminated soil (AMB100)			
MAGN	2.29 ab	2.24 bc	3.58 a
HEM	2.24 ab	2.16 c	3.37 a
BER	2.51 a	2.43 abc	3.54 a
SS	2.37 ab	2.64 ab	3.86 a
SSTBS	2.18 ab	2.39 abc	3.51 a
TBS	2.55 a	2.44 abc	4.07 a
BIRN	2.39 ab	2.13 c	3.83 a
HMOCaO	2.32 ab	2.31 abc	3.45 a
MAGH	2.25 ab	2.45 abc	3.39 a
Untreated	2.18 ab	2.74 a	3.72 a

Mean values with different letters within a column are significantly different ($p < 0.05$).

In contrast to bean, ryegrass growth was not affected by the Zn excess in untreated AMB100 (Tab. IV) and the smallest yield was even obtained in the control soil for every harvest. In the untreated and treated Zn-contaminated soils, nutrients are likely released as sludge progressively decays in the soil. The ryegrass growth for AMB100 was increased in cut #2 and cut #3. At the first and third harvests, changes in ryegrass yield among treatments were not significant. But in cut #2, magnetite, hematite and birnessite were less effective than the other materials.

3.3. Zn availability to plants

For the primary leaves and shoots of bean, the smallest Zn concentrations were found for AMB0 and BER-treated soils (Tab. V). The largest ones were observed upon addition of iron oxides such as hematite, magnetite, and maghemite. Relative to AMB0, Zn concentration in primary leaves increased about four-fold when plants were grown on these treated-soils. In addition to beringite, several amendments, i.e. SS, HMOCaO, TBS, SSTBS, and BIRN, proved to be effective in mitigating Zn accumulation in aerial plant parts. Larger Zn concentrations were determined in roots than in aerial tissues. The smallest Zn concentrations in roots were measured for the control soil and the beringite addition. Both birnessite and beringite reduced Zn uptake. But relative to the application rate, birnessite was the most effective to reduce shoot-Zn because it was only applied at 10 g·kg^{-1} whereas BER was used at 50 g·kg^{-1}. Beringite applied at 10 g·kg^{-1} was found uneffective in limiting Zn toxicity for dwarf beans in a Zn-contaminated soil [35].

Table V. Zn and Mn concentrations (mg·kg^{-1}, DW) in plant parts of 14-day-old *Phaseolus vulgaris* seedlings.

Soil treatment	Primary leaf		Shoot	Roots	Shoot/roots
	Zn	Mn	Zn	Zn	Zn
Control soil (AMB0)	44 e	58 d	56 d	430 g	0.13
Zn-contaminated soil (AMB100)					
Untreated	no plant		no plant	no plant	
TBS	94 cd	80 c	90 c	884 e	0.10
SS	86 cd	93 c	104 b	1152 cd	0.09
SSTBS	98 cd	87 c	93 bc	943 e	0.10
MAGN	171 a	428 a	143 a	1703 a	0.08
MAGH	143 b	269 b	139 ab	1473 b	0.09
HEM	134 b	90 c	138 ab	1297 c	0.11
BER	69 de	83 c	62 d	580 fg	0.11
BIRN	87 cd	299 b	82 c	695 f	0.12
HMOCaO	112 bc	464 a	96 c	1037 de	0.09
CaO	no plant		no plant	no plant	

Mean values with different letters within a column are significantly different ($p < 0.05$).
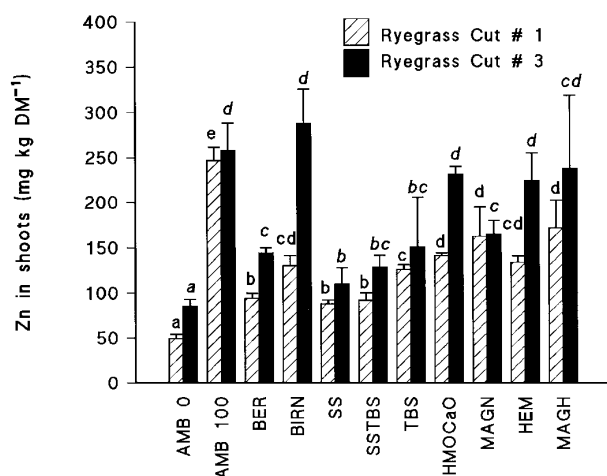
Figure 2. Zn concentration in ryegrass shoots harvested at 3 months (cut #1) and 5 months (cut #3) following a single application of amendment on a coarse sandy soil contaminated by sludge-borne Zn. For each cut, mean values with the same letter are not significantly different at the 5% level (Newman-Keuls test).

The sustainability of the Zn-immobilising effect following a single application of amendment was tested using subsequent ryegrass cuts from 2 to 5 months after the soil treatment. Zn concentrations in ryegrass shoots collected at the 1st and 3rd harvests are shown in Figure 2. Critical value (at a 20% reduction level) for Zn in ryegrass shoot was between 370–560 mg·kg DW^{-1} at soil pH 6.7 [22]. Zn concentration in the ryegrass shoot collected in untreated AMB100 was four to five times larger compared to AMB0 depending on the number of harvests. At the first cut, a decrease in shoot Zn concentrations was observed for all treated soils (Fig. 2). However, the most effective amendments for mitigating Zn uptake in shoots were SS, SSTBS and beringite; on the other hand, iron oxides (maghemite, magnetite, and hematite) as well as Mn-oxides (birnessite, HMOCaO) were the least effective treatments. The immobilising effect was further examined at the 3rd harvest (Fig. 2). Zn availability to ryegrass cultivated in AMB0 and AMB100 did not change relative to the 1st harvest. SS and SSTBS treatments were again among the most effective soil amendments leading to a decrease in Zn concentration in shoots. Beringite, basic slags and magnetite reduced also Zn

accumulation in the ryegrass shoots. The effect of birnessite addition on Zn availability was not persistent and Zn concentration in ryegrass shoots was similar for both the untreated and birnessite-treated soils. Zn sorption on Mn-oxides may have been affected by the roots potted in a relatively small volume. Roots of some plant species are able to release Mn from Mn-oxides in the rhizosphere [30], and inorganic elements may be recycled from disintegrated roots as well. Mn concentration was determined in ryegrass shoots harvested at the 1st and 3rd harvests (Fig. 3). Indeed, Mn concentrations were larger at the 3rd harvest than at the 1st one for all treatments investigated, and a very high Mn concentration was noticed in the HMOCaO treatment. The highest relative increase in Mn concentration (4 times) was found for the birnessite-treated soil. The subsequent ryegrass harvests preclude the birnessite effectiveness in this pot study but this must be tested using a larger soil volume and a longer growth period. Rhizosphere process and roots containment may also affect Zn sorption by SS, SSTBS, and BER and must be studied as well. At a zinc-smelter contaminated field site, the Zn water-extracted fraction was up to 70 times smaller in the beringite-treated soil 5 years after application of the soil amendment [35].
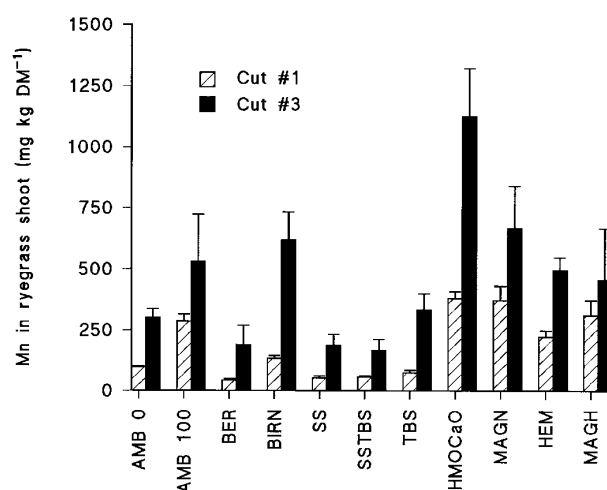

Figure 3. Mn concentration in ryegrass shoots harvested at 3 months (cut #1) and 5 months (cut #3) following a single application of amendment on a coarse sandy soil contaminated by sludge-borne Zn. For each cut, mean values with the same letter are not significantly different at the 5% level (Newman-Keuls test).
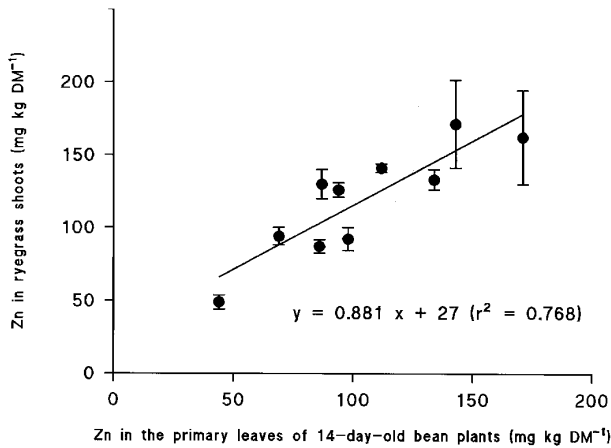
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Figure 4. Relationship between Zn concentrations in dwarf bean and ryegrass (cut #1) shoots for plants grown on the zinc-contaminated soil, untreated and treated by the addition of amendments.

Zn concentrations in the primary leaves of dwarf bean and ryegrass shoots (1st harvest) are linearly related (Fig. 4). Zn in ryegrass shoots harvested on the Zn-contaminated soil was 246 ± 15 mg Zn·kg^{-1} DM. Accordingly, the relationship allowed for estimates of Zn accumulation in primary leaves of dwarf bean as large as 249 mg Zn·kg^{-1} DM, thus exceeding the critical value in this bean part, i.e. 227 mg Zn·kg^{-1} DM [32]. This likely explains why bean plants did not grow in the untreated AMB100 soil.

3.4. Relationship between soil Zn extracted and Zn in aerial plant parts

Decrease in extractable Zn in soil following the application of amendments (i.e. BER, BIRN, SSTBS, TBS, HMOCaO) was mirrored by a decrease in Zn accumulated in both bean shoot and primary leaf (Figs. 5a and 5b). For the shoots, the combination of two Michaelis-Menten equations was found to be the best curve fit, with in a first plateau at 95 mg Zn·kg DM^{-1} corresponding to 15 mg Zn extracted by 0.1 M Ca(NO$_3$)$_2$ per kg soil DW. This may reflect subsequent mechanisms in bean as Zn exposure increased. Zn in ryegrass shoots at the first harvest and extractable Zn in soil are also related (Fig. 5c).
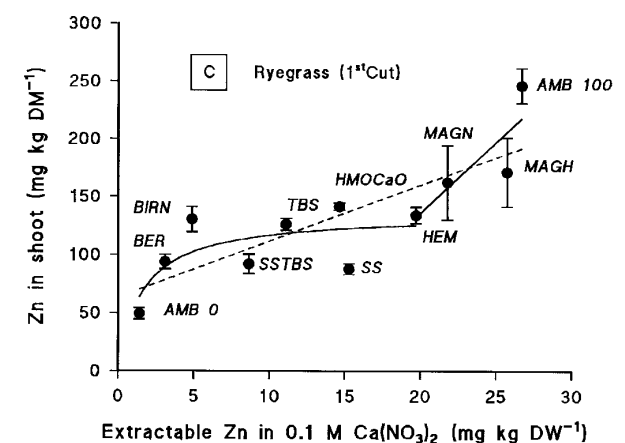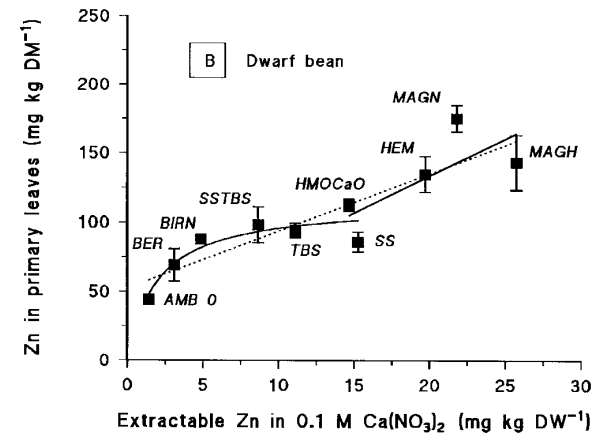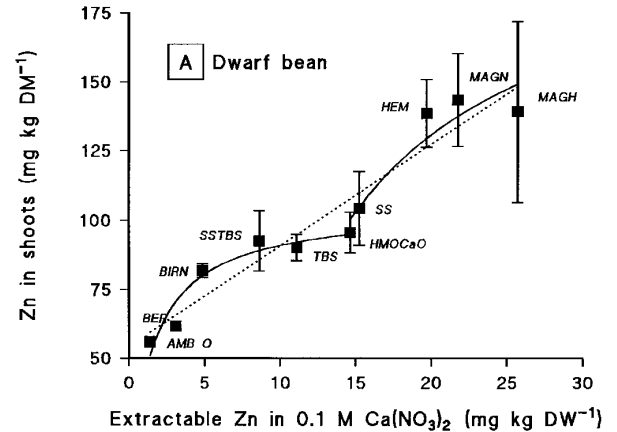

Figure 5. Relationship between Kd$_{Zn}$ (0.1 M calcium nitrate extractable-Zn vs. total Zn content in soil) and Zn concentration in the shoot (a) and primary leaves (b) of dwarf bean, and in ryegrass shoots (c).

3.5. Biological evaluation of the soil phytotoxicity

Metals assimilated through the roots interfere with metabolic processes. Early biochemical responses to metals, e.g. changes in enzyme activities, can be detected before the appearance of any visible symptom of phytotoxicity, and used as diagnostic criteria to quantify soil phytotoxicity [32, 33]. The induction of GPX, ME, ICDH, G6PDH, and GDH in the primary leaves of dwarf bean is considered to limit the detrimental effect of Zn on metabolic processes [31]. Therefore, these enzyme capacities and the soluble protein content were measured on the bean primary leaves. Most significant effects between the soil treatments were found for all these parameters when data were expressed per g fresh weight (FW) (Fig. 6). Soluble protein content was smallest in the HEM, BER, and SSTBS treatments (Fig. 6a); their values were similar to the control ones. Largest protein content was found for birnessite and HMOCaO.

Peroxidase induction is thought to be related to defense against oxidative stress, especially for scavenging the formation of hydrogen and organic peroxides, and is correlated to Zn, Cd and Cu assimilated by dwarf bean [32]. For control soils, typical values for GPX activity in primary bean leaf range between 900–2 000 mU·g FW^{-1} depending on season [26, 33, 34]. The value for AMB0 was within this range (Fig. 6b). Relative to the control soil, a significant increase in GPX activity occurred when plants were grown on the Zn-contaminated soil treated with HMOCaO, SSTBS, SS, and MAGN. GPX activity was restored to the control value by the following amendments: beringite, birnessite, and TBS. Induction in GPX activity was greatest for the HMOCaO treatment even though its leaf Zn concentration was lower than in MAGN (Tab. V), and an additional process was hypothesised. Leaf analysis showed a large increase in Mn concentration upon hydrous Mn oxides or magnetite addition to the soil (Tab. V), and Mn accumulation is supposed to induce GPX activity in bean [25]. Hydrous Mn oxide was synthesised at low pH and therefore soluble. CaO application was likely unsuccessful in inhibiting the HMO dissolution in soil.

In primary bean leaf grown on control soils, typical values for the G6PDH, ICDH and ME activities are 300–400 mU, 400–700 mU, and 600–700 mU per g FW^{-1}, respectively [26, 33, 34]. These enzyme activities increase as a result of Zn accumulation in these leaves [26, 31–33]. Significant changes in ICDH, G6PDH, and ME activities were measured among the treatments (Figs. 6c, 6d and 6e). For G6PDH, the addition of BER, BIRN, and SS to the zinc-contaminated soil resulted in an enzyme activity similar to the control value. In contrast, the addition of iron oxides such as maghemite, hematite and magnetite showed the largest Zn concentration in the primary leaves and thus G6PDH activity was markedly high. Beringite, and birnessite led to a decrease in both ICDH and ME activities, reaching typical values for control soils. This reflects the decrease in Zn concentration in primary leaf due to these treatments. Relationships between Zn concentration in the primary leaf and either ICDH, ME or G6PDH activity were evidenced (Fig. 7). The higher capacity of these enzymes which catalyse NAD(P)+ reducing reactions has been suggested to compensate for a possible shortage of reducing capacity in the cell because Zn inhibits chloroplast NADPH production [31]. GDH activity in the primary leaves of dwarf bean grown on control soils was 300–400 mU per kg FW. A significant increase in GDH activity was found only for the HMOCaO treatment (Fig. 6f). It was also reported for dwarf bean following HMO addition in a limed-silty soil [25], and may be related to increase in leaf Mn concentration. However, GDH induction was not found for MAGN even though the leaf Mn concentration was similar. Critical value for Mn toxicity in dwarf bean leaf, i.e. 1 000 mg·kg^{-1} DM [22], is not exceeded (Tab. V).

Even though changes in shoot length and dry-matter yield in dwarf bean were unsignificant among soil treatments except for limed and untreated soils (Tab. III), metabolic responses in the primary leaf allow two treatment groups to be distinguished. Birnessite (10 g·kg^{-1}), and beringite (50 g·kg^{-1}) applied to the Zn-contaminated soil clearly restored the enzyme activities in the primary leaves of 14-day-old plants to control values. This
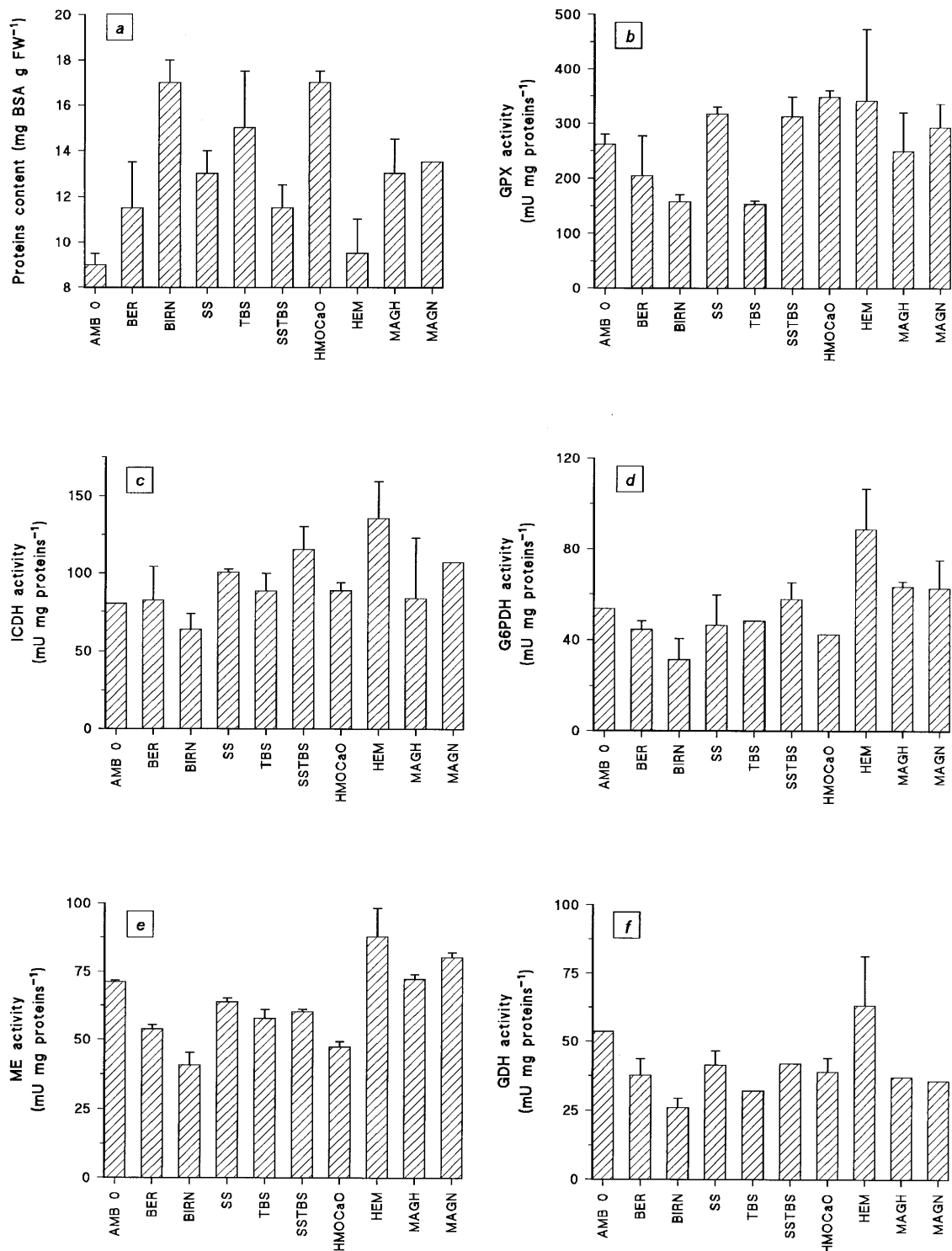
Figure 6. Soluble protein contents (a), (gaïacol)peroxidase (b), isocitrate dehydrogenase (c), glucose-6-phosphate dehydrogenase (d), malic enzyme (e), and glutamate dehydrogenase (f) activities in the primary leaf of 14-day-old *Phaseolus vulgaris* L. grown on the control soil, the untreated and amended Zn-contaminated soils.
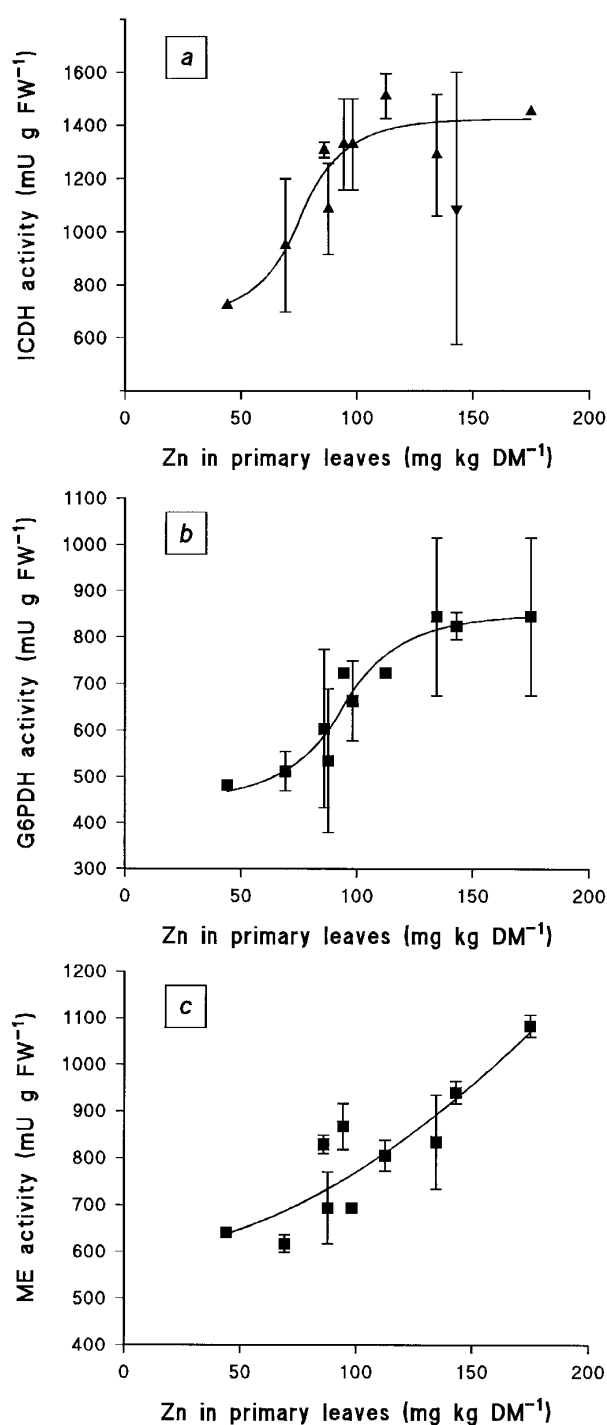
Figure 7. Changes in isocitrate dehydrogenase (a), glucose-6-phosphate dehydrogenase (b), and malic enzyme (c) activities in relation to Zn concentration in primary leaves of 14-day-old *Phaseolus vulgaris* L. grown on the control soil, the untreated and amended Zn-contaminated soils.

reflected the decrease in Zn accumulation in primary leaves (Tab. V). Application of hematite, steel shots with and without basic slags, basic slags, maghemite, magnetite, HMO combined with lime in the zinc-contaminated soil did not suppress the metabolic stress due to Zn assimilated from the soil.

4. Conclusion

This pot experiment aimed to evaluate the amendment effect on sludge-borne Zn phytoavailability in a sandy soil. The range of inorganic compounds comprised iron oxides (hematite, magnetite, maghemite), manganese oxides (birnessite, hydrous Mn oxides), steel shots that oxidised in iron and manganese oxides, a modified aluminosilicates (beringite) and alkaline compounds (lime, Thomas basic slags). Based on the addition rate, the largest decrease in extractable Zn was found in the birnessite (10 g·kg^{-1})-treated soils. Steel shots combined with TBS were the second promising amendment. Beringite was very efficient but with a higher addition rate (50 g·kg^{-1}). It is suggested that an alkaline material such as beringite combined with steel shots may hold promise to immobilise Zn in soil. Products such as beringite, steel shots, and basic slags are even more interesting because they are commercially available in industrial quantities. Germination of dwarf bean was highly affected in the untreated and limed zinc-contaminated soils, whereas all other amendments restored and enhanced the growth of 14-day-old dwarf bean. Zn concentration in bean aerial parts was decreased and enzyme activities in primary leaves restored to the highest extent with beringite and birnessite. Amendments such as SS, SSTBS, TBS and HMOCaO were also efficient to limit the root exposure to Zn and consequently its accumulation in bean aerial parts. Similar results were found for the first cut of ryegrass shoots. Based on subsequent harvests of ryegrass, decrease in Zn availability as a result of either beringite, steel shots, steel shots combined with basic slags, or basic slags addition was persistent for a 5 month period. Although pot experiments cannot replace long-term field experiments, this result suggests that the effect of these

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amendments on sludge-borne Zn phytoavailability can be sustainable. In a field experiment at the site of a former Zn smelter in Belgium, the metal immobilising effect of a beringite soil treatment (50 g·kg^{-1}) remains effective over at least a 5 year period [34, 35]. To further test available products such as beringite and steel shots in practice, they were applied at 50 g and 10 g·kg^{-1} into the top layer (0 to 25 cm depth) of four experimental plots of a field experiment with sludged-soils at the INRA Couhins experimental farm, Bordeaux, France. The long term effect of these soil treatments is being studied with a maize monoculture. In contrast, reduction in Zn availability to ryegrass by birnes-site was not sustainable on a 5 month period. It is recommended that further long-term field trials be performed on the addition of beringite, steel shots, basic slags and their combinations, with various soil types and anthropogenic zinc origin. Further experiments are needed at various concentrations and frequencies of soil application of birnessite before its long term efficacy can be evaluated.

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