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Plant diseases caused by heavy metals and their phytiatry with cation exchangers

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Abstract

The noxious influence of heavy metals as lead, zinc, copper and lead on higher plants and soil fungi was described. Addition to the soil of Levatit cation exchangers consisting of polystyrene porous resins, charged with calcium or manganese in granulated or powdered form, restored normal plant growth and inhibited the uptake of heavy metals ions.

I. INTRODUCTION

In phytopathological investigations it is usually to look first for the causes and conditions responsible for the abnormalities. Plant diseases are caused not only by parasitic organisms but also by abiotic conditions as lack of nutrients and excessive salt concentrations.

In as far as industry, agriculture and horticulture are carried on in close proximity, questions are bound to arise — even when vigorous steps are taken to prevent pollution — about the effects of pollution or plant development.

Throughout the world a great deal of work is being done on one of the relevant problems: that of heavy metal pollutants, their sources and distribution. Adverse effects of heavy metals on plant development have been reported again and again over a long period. As awareness of the environment has increased, more and more, attention has also been paid to other factors responsible for adverse effects on plant growth, as well as to their prevention and cure.

In our study of soil contamination as a cause of plant diseases we have been concerned particularly with the heavy metals lead, copper zinc, especially in as far as they occur in the enviroments of industry or in heterochthonous substrates used in the cultivation of pot plants.

II. INFLUENCE OF HEAVY METALS ON PLANT LIFE

1. Influence on the development of macroflora

The favourable effects of heavy metals, such as copper and zinc, are regarded by several scientists as connected with their functions in enzyme activity and plant growth regulation. But excessive concentrations in the soil are toxic to plants as may be seen from poor germination, cryptobiosis, chlorosis, and necrosis of roots and leaves.

The concentrations at which heavy metals in the soil become toxic to flora and fauna are difficult to state exactly. They depend on the plant species, the cation exchange capacity of the soil, plant roots, and their possible interaction. The absorption and toxicity of the heavy metals depend partly on such properties of the soil as the mineral contents, concentration of colloids, content of organic mater, acidity, moisture, and concentrations of nutrients and salts. They also depend on such botanical factors as the plant's state of activity, physiological development and on whether or not the plant is a dicotyledon or monocotyledon.

Copper appears to have little mobility and to be highly phototoxic. It causes blackish brown discolouration of roots, which often develop a syndrome commonly known as "barbed wire". The copper concentration is non-injurious, though not necessary normal, if it is below 40 ppm (parts per million dry soil weight; Cu in the nitric acid, 1 N extract) (de la Lande Cremer 1976).

Lead in the soil is a cause of plant diseases only if its concentration there is relatively high (Henkens 1975). In particular, lead is believed to depress transpiration and photosynthesis. This effect appears to be connected with the progressive resistance of the stomata (Bazzaz 1974). Zinc has a middly antagonistic effect, e.g. on iron, and is toxic at concentrations of 125 to 500 ppm, determined by extraction with acetic acid (Henkens 1975).

So far, little is known about the complex and specific effects of these metals. Interactions between them are therefore possible when more than one of them is present simultaneously.

2. Contamination of human and animal foods

Conclusions about the toxicity of lead, copper and zinc to humans differ considerably from author to author. In Belgium, as in many other countries, there is still no legislation on the amounts of heavy metals which may be present in and on food and vegetables. The figures about to be given are intended to indicate the orders of magnitude of permitted or proposed concentrations.

In the Federal Republic of Germany a bill has been published which limits the lead content of foods. The highest permissible lead concentrations are laid down as 0.5 ppm (calculated on fresh weight) for lettuce, cabbage and root vegetables, and 1.0 ppm for other vegetables (excluding mushrooms) and fruit (Pfeilsticker 1975).

The average amount of copper ingested by Europeans with their food is 3.1 mg per kg of food. No accumulation occurs in the human organism if this ingestion rate is not exceeded (Lageveen 1972). In Belgium a ministerial orded of December 6, 1968 restricted the copper content of fruit and vegetables to 20 ppm (as Cu) and 50 ppm for celery leaves.

Zinc, unlike lead, is an essential nutrient, of which the human requirement is 6 mg a day (Michels 1974). But this amount may be greatly exceeded. In Canada the Food and Drug Act Regulations (1970) lay down a maximum concentration of 50 ppm (calculated on fresh weight).

3. Effects on the plant —parasite relationship

Some aspects of the adverse and toxic effects of heavy metals on plants, humans and animals have been mentioned above. In view of the very close relationships between soil microflora, soil fertility and plant growth it was appropriate to consider how heavy metals influence microorganisms of the soil and their ability to infect plants.

Rühling and Tyler (1973) have shown that, as the concentrations of heavy metals risse, there is a drop in the general decomposition rate of organic matter, such as spruce needle litter, measured as liberated CO_2 or dehydrogenase activity. Tyler (1974) investigated the efficacy of hydrolytic soil enzymes in humus contaminated with copper and zinc. Broadly speaking, straight regression lines are obtained between enzymatic activity and the concentration of copper and zinc, while highly negative coefficients are obtained for urease and acidic phosphatase.

According to Schönbeck (1974) the number of bacteria is strongly reduced. Decomposition of cellulose is inhibited and, in some cases, completely prevented. *Azotobacter,* which is an indicator of nitrogen-fixing microorganisms, and also green algae can decrease to the zero level. The percentage of diatoms falls practically to zero, and that of the spore-forming fungi and actinomycetes may be greatly reduced.

Among the microflora of the soil, soil fungi are very important, partly because of their role in the decomposition of organic matter and partly because of their ability to act as pathogens. Brief reference must therefore be made to the ways in which they are affected by heavy metals.

It is well known that copper salts have fungicidal effects and are used as fungicides. The average physiologicol toxicity of metal ions is in the decreasing order: Ag, Hg, Cu, Cd, Cr, Ni, Pb, Co, Zn, Fe, Ca. The toxicity in a particular case depends on the species of fungus, the nature of the accompanying anions and the nature the substrate. Organic chelates are thus more toxic than the metal ions themselves, provided that they are split up in the cell (Horsfall 1956).

The fungistatic effects of cations depend on the strength of their covalent bonds with organic groups of the cell (such as imidazole, carboxyl, phosphate and sulphydryl groups) (Somers 1961). The degree of toxicity is also govarned by the ability of the ion to penetrate the permeable barriers of the cell membranes (Cochrane 1958).

Inhibiting effects of heavy metals on spore germination are also very important. Copper, zinc, and cadmium reduce spore germination after only a few hours of contact (Miller and Mc Callen 1957). The spores, for their part, are able to accumulate heavy metals up to high concentrations. This accumulation can be explained partly as the result of a simple ion exchange process, e.g. with H^+, K^+. Mg^{++} and Ca^{++}.

In view of the facts and possibilities indicated above the phytopathologist must pay particular attention to the effects of soil-heavy metals on the relations between plants and parasites. The main factors on which these depend are the influence on the microflora and the development and infection capacities of the microorganisms, on the one hand, and together the proneness of the plant to necrosis of the roots and states of debility, on the other.

III. OPPORTUNITIES FOR TREATMENT OF SOILS WITH ION EXCHANGERS

1. General

In the last decade numerous investigations into the biotic infection potentials of soil organisms to plants have been carried out at the Laboratory of Phytopathology and Plant Protection of Louvain University. Studies to investigate the affectiveness of phytomedicinal processes, such as soil desinfestation, e.g. by chemical means using methyl bromide, have been conducted also. Soil desinfestation with methyl bromide, as a preventive and selective method of biological control with a broad spectrum of action is preferable to the regular and cumulative use of exogenic chemicals during cultivation itself. But the possible adverse effects of a bromide residue in the soil, which may be easily absorbed by plants, must be taken into consideration.

The results of numerous investigations suggest that absorption can be greatly reduced by the use of anion exchangers.

Analogous to soil infestation and soil desinfestation versus undesired or harmful biotic and abiotic entities and their interaction the research has been oriented to cation exchangers versus the considered heavy metals.

In the experiments about to be described we used Lewatit-cation exchangers. These consisted of macroporous resins based on polystyrene having slightly acidic functional groups charged with calcium or manganese and showing selectivity towards bivalent metal ions, e.g. lead, copper and zinc. They were used as granules or powders.

At the start of each test the heavy metals in the substrate were identified and their amounts were calculated in equivalents. The equivalent amounts of the cation exchangers (based on the exchange capacity in equivalent) were then either, in the case of autochthonous plots, introduced into the top 10-cm layer of the soil before sowing of annual cultures, or, in the case of heterochthonous substrates, completely mixed with the substrate.

2. Effects on macroflora

2.1. Microtest

For each exploratory investigation, and prior to each soil test, we performed tests on plants grown in small transparent plastic dishes in a greenhouse, that is to say under conditions which ensured that there

Table 1

Development ratings and residues of *Lepidium sativum* grown in a sandy soil (BE I) and in coniferous forest soil (BE III)

Treatment	pH	Root development rating (0—5)	Leaf weight as percentage of control	Residues in ppm, calculated on fresh leaf weight		
				Pb	Cu	Zn
BE I						
Control	5.8	0	100	89.7	225.0	197.0
Control+lime (1 kg/100 l)	6.9	0	133	20.4	25.1	41.6
Control+lime (250 g/100 l) +Lewatit Ca powder	6.8	5	145	9.9	17.5	18.2
BE III						
Control	4	0	100	18.5	20.9	21.0
Control+lime (250 g/100 l)	6.2	2	108	3.6	6.4	16.5
Control+lime (100 g/100 l) +Lewatit Ca powder	5.2	5	147	5.7	6.9	13.4

Heavy metal contens of the dry soils in ppm

	Pb	Cu	Zn
BE I	602	1221	537
BE III	1061	1113	178

was no further contamination. Garden cress (*Lepidium sativum* L). and peas (*Pisum sativum* L.) were used for most of the tests.

After no more than a week these micro-tests allowed to assess the application and quantity of the cation exchangers.

Table 1 shows the beneficial effects of cation exchangers on two substrates to which were added the equivalent amounts (calculated on the contaminating elements) of cation exchangers with an exchange capacity of 1.65 eq/l.

2.2. Fields trials

The biotests *in vitro* and in the greenhouse were followed in 1975 by a field trial on a sandy soil (BE XII) subject to continuous pollution. The concentrations of contaminants in the soil were 250 ppm for lead, 600 ppm for copper and 150 ppm for zinc. The results obtained on the control plot (I) were compared with those obtained on four other plots separately treated with the equivalent amount of Lewatit Ca powder (II), half of this amount of the same powder (III), an equivalent amount of Lewatit Ca powder and Lewatit Mg Powder in the ratio 50:50 (IV) and an equivalent amount of Lewatit Ca in granule form (V).

The treatment of each plot requiring treatment was effected in a single operation by introducing the exchanger or exchangers into the top 10-cm layer of the soil, after which all the plots, which measured 2.5×2.5 metres, were immediately sown with seed or planted with young plants seventeen different crops. It is reasonable to assume that the ecological conditions during the vegetation period were highly unfavourable. The plots had been unused for many years and showed little sign of biological activity, to some extent supporting *Agropyron repens* (couch grass) only. The initially very warm and dry weather affected the kinetic processes in the soil and the opportunities of the plants for development unfavourably, in addition to which pollution of the air continued without interruption.

As the original pH value of the soil was 5.0, all the plots were treated with lime (80% $CaCO_3$+8% $MgCO_3$) at the rate of 10 kg per are, with the result that the average pH value one month later was 6.4, with a maximum difference of 0.25 pH.

At regular intervals the plants on the plots were examined and samples from the plants were taken for quantitative evaluation and residue analysis. The plant growth ratings are presented in Table 2.

Leeks (*Allinum parrum* L. cv. Elboeuf), cucumbers (*Cucumis sativus* L.) and african marigolds (*Tagetes erecta* L.) were transplanted with pressed soil balls, and celery (*Apium graveolens* L. cv. goudgele chemin), salvias (*Salvia splendens* L.) and "Frigo" strawberries (*Fragaria virginiana* Ehrh.) without soil.

Table 2
Average ratings 0÷5 of plots under various crops

Species	I	II	III	IV	V
Petroselinum sativum Crantz.	0	3.25	1.5	4.5	0.25
Anthriscus cerefolium Hoffm.	0	3.5	1.25	3.5	0.25
Raphanus sativus L. cv. roodkopje.	0.5	4.5	3	4.5	1
Daucus carota L. cv. Beukummer.	0	4	1.25	5	0.75
Cichorium intybus L.	0	4	2.5	5	0
Festuca rubra L. cv. Bargarla.	1.5	4	2.75	5	0.25
Poa pratensis L. cv. Baron	2	5	4	5	2.50
Phaseolus vulgaris L. cv. Flageolet Holland select.	1	3.5	2.5	4.5	2
Allium porrum L. cv. Elboeuf	1	3.5	2.5	4.5	2.75
Cucumis sativus L.	1	3	2	4.5	1.25
Tagetes erecta L.	1	4.5	2.75	5	1.75
Apium graveolens L. cv. goudgele chemin.	0.25	3	2	4	1.75
Salvia splendens L.	0.5	4	2.75	4.5	1.50
Fragaria virginiana Ehrh.	0.25	4	2.5	4	1.25
Average	0.65	3.83	2.37	4.53	1.37

It will be seen from Table 2 that the average improvement in plant growth obtained by the use of cation exchangers is very remarkable. It is reasonable to assume that the improvements in quality and quantity, which are important profitability factors, differ much more from case to case than do the improvements in plant development.

The best average results were obtained with the mixture of cation exchangers charged with Ca and Mg (IV). The improvement obtained on plot II was, logically, much greater than that obtained on plot III, for which the amount of exchanger used on plot II was halved. It will be seen that granules (V) are less effective than powder (II), despite the fact that both have theoretically the same exchange capacity. The reason for this is that the difference in structure between the granules and powder gives the latter a higher exchange capacity in practice. In Table 3 the yields for two grass species, each mown three times, are shown individually and altogether.

At a later stage of the trials the field in which the plots were situated was sprayed with water regularly owing to the dryness of the weather. It is noteworthy that *Agrypyron repens* (couch grass) sprouted from the lower layers of the soil in the control plots, but not in the plots treated with the theoretical amounts of ion exchangers.

Experience gained in the trials showed that this indicator plant is interesting in other ways. Couch grass dies down annually in the topmost layer of the soil, leaving behind dry fragments which make it difficult to incorporate the ion exchanger. This may cause germination

Table 3

Absolute and percentual yields (in g/m²) for the lawn grasses *Festuca rubra* (FR) and *Poa pratensis* (PP), cut at three different dates

Treatment	Grass species	Yield in g/m² — Number of days after sowing: 62	82	119	Yield from m² in g	Yield from m² in %
I. Control	FR	46	103	206	355	100
	PP	62	186	527	775	100
II. Eguivalent weight Lewatit Ca powder	FR	799	715	1205	2719	760
	PP	660	745	1283	2688	347
III. Half equivalent weight Lewatit Ca powder	FR	543	406	971	1920	541
	PP	207	399	659	1265	163
IV. Equivalent weight Lewatit Ca+Mg powder	FR	1325	615	1086	3026	852
	PP	698	734	807	2239	289
V. Equivalent weight Lewatit Ca granules	FR	441	539	606	1686	475
	PP	340	608	1089	2047	264

difficulties, particularly when the seeds are small, like those of parsley (*Petroselinum sativum Crantz*) and chervil (*Anthriscus cerefolium Hoffm.*).

2.3. Heterochthonous substrate

Particularly in the cultivation of ornamentals, many heterochthonous substrates are used, as such or mixed with other substrates. Soil from coniferous forests, certain types of peat and waste water sewage can be mentioned as examples. According to their origin, they may be contaminated with heavy metals, and they may be toxic to plants, depending on the species and state of development. Trials have been conducted with rooted azalea (*Azalea indica* cv. Ambrosiana) cuttings and with cuttings and seeds of various ornamental plants.

The results of a test on young seedlings of *Dizygotheca elegantissima* grown in coniferous forest soil BE III may be given as an example. According to the degree of contamination and specific gravity of the soil and exchange capacity of the ion exchanger, the theoretical requirement of 1.5 kg of cation exchanger, consisting of 50% each of the Mg and Ca forms, was added as a fine powder to each cubic metre of soil.

The effects of the heavy metals and cation exchangers on the plant development — adverse in the former case and beneficial in the latter — were assessed according to botanical criteria. The average rating achieved by the plants on the control plots was 1.5, whereas the highest rating, 5, was achieved by the plants on the treated plots.

In particular it was found, that, in addition to being adversely effected by soil contamination, the germination of seeds and rooting of mainly herbaceous cuttings may be affected very favourably by the inactivation of undesirable trace elements.

3. Effects of ion exchangers on residues

In addition to improving plant development and plant yields, the inactivation of heavy metals by cation exchangers greatly increases the wholesomeness of edible plants.

The results of tests on garden cress will be found in Table 1. Identical heterochthonous substrates were also used for radishes, spinach and beats (Table 4).

Table 4

Residue analyses of spinach grown in contaminated soil (BE I=602 ppm Pb, 1221 ppm Cu, 537 ppm Zn), untreated and treated with cation exchangers

Treatment	Content in ppm, calculated on fr. wt.			Leaf weight %
	Pb	Cu	Zn	
Control	83.2	246.5	698	5
70% / equivalent weight Lewatit Mg powder	2.5	7.8	106	100
70% / equivalent weight Lewatit Ca powder	1.7	4.4	38	125

One should not generalize these results in practice, partly because the soil of the plots was very heavily contaminated and partly because, for that reason, the plants on the control plot remained exceptionally small, with the result that their residue concentrations were very high.

Corresponding to the effective results of plant development, the enhanced reduction of the residue content or the absorption of heavy metals, by the use of cation exchangers is very illustrative.

A review of the effects of soil residues on the roots, fruits and leaves of plants grown in the above-mentioned field trials BE XII, which were exposed without interruption to polluted air, is given in Table 5.

It will be seen that the binding of heavy metals by cation exchangers caused a big reduction in their absorption by the plants. A remarkably high degree of sensitivity to polluted air was shown by the leaves of chervil.

Table 5

Residue analyses (in ppm, calculated on fresh weight) of vegetables grown outdoors on severely contaminated plots (BE XII), untreated and treated with ion exchangers and exposed without interruption to polluted air

Treatment	Washed with water						Unwashed chervil		
	radish roots			strawberry fruits					
	Pb	Cu	Zn	Pb	Cu	Zn	Pb	Cu	Zn
I. Control	5	16	110	<1	7	14	29	174	267
II. Equivalent weight Lewatit Ca powder	<1	2	11	<1	4	4	16	58	54
III. Equivalent weight Lewatit Ca+Mg powder	<1	<1	9	<1	4	5	29	113	180

4. Effects on microflora and the plant-parasite relationship

As mentioned above, the microorganisms of the soil are affected in many ways, and very strongly, by the presence of heavy metals. In this connnection it is interesting to consider the following:

1. How exchangers influence the population of saprophytes and potential parasites in the soil.

2. How the exchangers affect the relationship between harmful organisms and the roots of cultured plants that are suffering from necrosis caused by heavy metals.

The development of the bacteria, fungi and actinomycetes populations was observed in a sand substrate (BE XII) with and without additions of cation exchangers. The populations after three months are shown in Table 6.

Broadly speaking, the results indicate that contamination of the soil with heavy metals reduces the microbial activity. Conversely, the microbial activity increases when the heavy metals are blocked. As the effects of heavy metals and cation exchangers are thus diametrically opposed, it is interesting to consider how the exchangers affect such processes and properties of the soil as biodegradation, antagonism and infection of plants.

Table 6

Population densities of microorganisms in soil contaminated with heavy metals, untreated and treated with exchangers. Organisms per gram dry soil

Treatment	*Bacteria* $\times 10^3$	*Fungi* $\times 10^3$	*Actinomycetes* $\times 10^3$
Control	950	32	41
Equivalent weight Lewatit Ca powder	1940	40	83
Equivalent weight Lewatit Ca+Mg powder	2490	60	111

Table 7

Average development (in mm/day) of some fungus species in test tubes filled with soil contaminated with heavy metals, untreated and treated with cation exchangers

Treatment	*Tricho-derma*	*Rhizoctonia*	*Fusarium*	*Pythium*
Control	0.54	0	0.08	0
Equivalent weight Lewatit Ca powder	0.67	0.15	0.30	1.41
Equivalent weight Lewatit Mg powder	0.72	0.55	0.23	1.57

The development of four species of fungus in the same soil (BE XII) is shown in Table 7.

Apart from antagonistic value of some soil fungi, as *Trichoderma*, the increase of the development of the entities with parasitic potency could be an indication for higher plant pathogenous effects.

It is important to note that the relationship between the plant and parasite, and the etiology of the infection, may differ from case to case. In many cases, as with *Rhizoctonia*, the pathogenesis depends very much on the population density, even when the host plant has not been typically weakened. With other fungi, such as *Verticillium* and *Fusarium*, which must be classed as typical wound parasites, the state of necrosis of the roots indicates theoretically an increased risk of infection of the plants. But immobilization of the heavy metals closes this infection route, a fact which appears to explain the improvement in plant resistance.

On the other hand pectin lyase is produced by *Rhizoctonia solani* and *Fusarium solani*. This enzyme is stimulated by calcium ions and its activity depends on the degree of esterification of the substrate and the acidity of the reaction mixture (Sherwood 1966; Batemann 1966; Voragen et al. 1971). Apart from the fact that the decontamination of heavy metals in the soil neutralises the toxicity it is questionable whether the infection potency is not enhanced by the activity of the exchanged cations, especially calcium.

In our opinion a more detailed investigation of the effects of heavy metals on the plant-parasite relationship could be very rewarding. The more so as the cation exchanger is not applicated as in the classical phytopharmaceutical context against undesired organisms but within the whole of the relation between plants and parasites.

IV. REMARKS AND CONCLUSIONS

There is no doubt that such heavy metals as lead, copper and zinc may be toxic to microorganisms, plants, animals and humans, when present in the soil. The harmfulness of anyone of these element depends on the genotypes and phenotypes of the microflora and macroflora. The occurrence of cryptobiosis, chlorosis, dying-óff, necrosis, residues and increased pathogenesis is very common in plants grown in polluted soils or in heterochthonous substrates taken from polluted areas.

Addition to the soil of cation exchangers, in equivalent quantities calculated on the degree of contamination, is capable to a significant extent of normalizing plant development and reducing the plants take-up of heavy metals. Further investigation of the effects of cation exchangers on biological factors, including the plant-parasite relationship, appear particularly desirable.

Blocking of those nutrient that are normally desirable has not yet been observed, either in the greenhouse or outdoors. The structures and charges of the cation exchangers considered are such that there is no risk to monovalent nutrient cations. As far as bivalent elements are concerned, there is likewise no risk to manganese. Theoretically, there could be a risk to iron; no adverse effect on iron has yet been observed; however apart from the typical picture seen on the control plots, which was due to the antagonistic effect of free heavy metals.

There is no doubt that the use of agricultural chemicals has improved both the quantity and quality of crops. Our experiments have shown that the use of agricultural chemicals does not result necessarly in the formation of "additional resideus", but in the fixation of abiotic entities undesired in ecological or biological environment.

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Choroby roślin wywołane ciężkimi metalami i ich leczenie wymiennikami kationowymi

Streszczenie

Opisano ujemne działanie ciężkich metali — takich jak cynk, miedź i ołów — na grzyby glebowe i kilka roślin wyższych.

W celu usunięcia szkodliwych efektów metali ciężkich wprowadzono do gleby wymienniki kationowe Lewatita złożone z porowatych polistyrenowych żywic z grupami funkcjonalnymi naładowanymi wapniem lub manganem, w postaci granulowanej lub sproszkowanej. Powodowały one przywrócenie normalnego wzrostu roślin i hamowały pobieranie przez rośliny jonów ciężkich metali.