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ORIGINAL ARTICLE

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Reduction of aluminum toxicity to radish by alkaline oxygen treated kraft lignin

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Abstract To obtain a soil-conditioning agent for acid soil containing excess aluminum ions (AL), kraft lignin was modified by alkaline oxygen treatment. The growth of radish root in solution and in soil containing AL with or without addition of these lignins under controlled pH was examined. We concluded the following. Growth inhibition of radish roots by AL can be removed by adding alkaline oxygen-treated lignins in the range of pH 4.5–4.8 in soil. A similar result was obtained at pH 4.5 in a culture solution. The reduction of AL toxicity to plant may be due to the aggregation between AL and the modified lignin at low concentrations of modified lignin because soluble AL could not be detected. On other hand, elongation of radish root was not obviously inhibited, although the soluble AL in the solution culture was at an extremely high level when the dosage of a modified lignin was high. This suggests that the reduction in AL toxicity to plants was due to formation of a complex between AL and acidic groups of the modified lignin.

Key words Acid soil · Aluminum ions · Alkaline oxygen treated lignin · Soil-conditioning agent · Solution culture

Introduction

Acidification of soil is well known to cause poor plant growth, and one of the main reasons is believed to be the elution of aluminum ions (denoted as AL) from soil matrix below pH 4.5. It is widely reported that plant growth is inhibited seriously by AL.[1–3] Aluminum attacks plant roots and stops their elongation.[4] Plants consequently cannot take up nutrients necessary for their growth, and they finally die.

In a previous study[5] we prepared a radical sulfonated lignin and alkaline oxygen-treated lignins from a commercial softwood kraft lignin. A sulfonic acid group was introduced, probably at the *ortho* position, to a phenolic hydroxyl group in lignin by radical sulfonation treatment.[6] Carboxylic acid groups were introduced by a ring-opening reaction of aromatic nuclei during alkaline oxygen treatment.[7] These functional groups were expected as ligands to combine with metal ions (i.e., toxic AL) in acidic soil.

Modified lignins could remove the toxicity of AL to the growth of radish (*Raphanus sativa* var. *radicula* Pers.) root. Growth of radish root in a sandy soil (Toyoura sand) was directly evaluated by neutron radiography.[8] Although the growth rate of radish root was improved in the presence of a modified kraft lignin, it was not clear which was the major effect for the reduction of AL toxicity: the pH increase by addition of a modified lignin or the formation of an AL–lignin complex. It is possible that radish growth is inhibited by the acid itself; the other possibility is different inhibition depending on the degree of hydrolysis of aluminum salt and polynucleation of AL due to the increased pH of the solution.[9–11] In this case insoluble, nontoxic AL species may increase in culture solution.

We focused here on the nature of the AL–lignin complex to evaluate modified lignins as soil-conditioning agents. The growth of radish root in solution and in soil containing AL with or without addition of alkaline oxygen treated lignins under controlled pH conditions was examined.

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Materials and methods

Alkaline-oxygen treatment of industrial kraft lignin

Six grams of a commercial softwood kraft lignin purified by an aqueous dioxane/diethylether system was dissolved in

Table 1. Sample preparation conditions and amount of acidic groups in modified lignins

Sample	O_2 pressure (Mpa)	Temp (°C)	Time (h)	Type of treatement	COOH[a] (eq./200 g lignin)	-PhOH[b] (eq./200 g lignin)
L4	0.3	70	4	Alkali-oxgen	0.31	–
L8	0.3	70	8	Alkali-oxgen	0.40	0.24
KL	AP	RT	–	Control	0.22	–

AP, atmospheric pressure; RT, room temperature
[a] Measured by pH titration
[b] Measured by the $\Delta\varepsilon i$ method

0.1 M NaOH (300 ml) and treated in a glass autoclave (500 ml volume; TEM-U, Taiatsu Techno) under the conditions shown in Table 1. Oxygen pressure was kept constant at 0.3 MPa throughout the treatment. For the preparation of a control sample (KL), an alkaline solution of kraft lignin was kept in air at room temperature. Reaction mixtures were transferred to cellulose tubes (UC 36-32, Viskase Sales Co.) and dialyzed against deionized water for 3 days. The solution was then evaporated to dryness in vacuo and used for further studies.

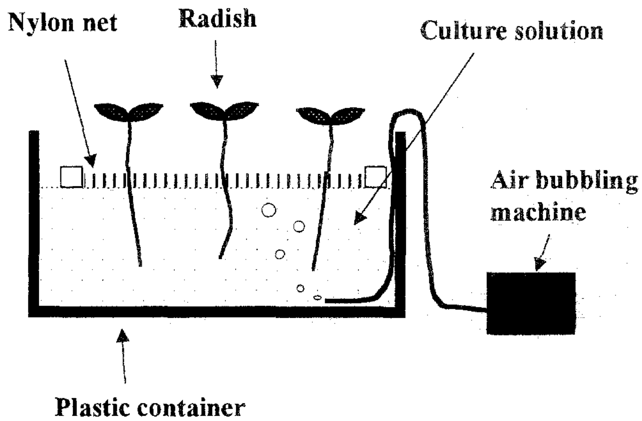

Fig. 1. Growth system of radish by culture solution

Functional group analysis of modified lignins

A modified lignin was treated with a cation-exchange column (IR-120B) and was evaporated to dryness. A free acid type modified lignin (50 mg) was dissolved in 30 ml of methyl cellosolve/water (1:19) and was used for the determination of carboxyl groups by potentiometric titration. Nonconjugated phenolic hydroxyl groups were determined by the $\Delta\varepsilon i$ method.

Efficiency of modified lignins to remove AL toxicity in soil

Radish was used as the test plant. Radish seeds were treated with a gibberellin solution overnight and then germinated on a filter paper soaked in deionized water in a controlled room under natural light. After 3 days, young seedlings were transferred to an Aluminum container (16 × 25 × 0.3 cm) coated with a Teflon sheet. Toyoura's standard sand containing 16% water in the sand was packed in a container. Aluminum sulfate and Hyponex in water (180 ppm and 2%, respectively) were added to the water. Diluted sulfuric acid and potassium hydroxide were used to adjust the pH. Containers were kept in a room in which the temperature was controlled at 25°–30°C under natural light. Ten days after transplantation the radish root length was measured by the line intersection method.

Preparation of nutrient solution

Malkanthi's method[12] was used to prepare solutions for cultivation in a 2-l plastic container. The concentrations of nutritients in the solution were as follows: 0.6 KNO_3, 0.04 KH_2PO_4, 0.2 $NaNO_3$, 0.4 $CaCl_2$, 0.2 $MgSO_4$ (in millimoles); and 4.0 Fe, 6.0 B, 1.0 Mn, 0.1 Zn, 0.06 Cu, and 0.02 Mo (in micromoles). $KAl(SO_4)_2 \cdot 24H_2O$ was added as the Al source. The AL concentration was varied from 0 ppm to 50 ppm. A calculated amount of an alkaline oxygen treated lignin (L4) was added to the culture solution as an aqueous solution. The solution was adjusted initially to pH 4.5 by diluted aqueous nitric acid and potassium hydroxide; it was readjusted twice a day to keep the pH constant.

Determination of AL concentration in culture solution

A culture solution was centrifuged (at 14000 rpm, 10 min). The supernatant was used to determine the AL concentration by the Aluminon method.[13]

Measuring the elongation rate of the primary root

Radish seeds were germinated on a filter paper soaked in deionized water for 4 days. Ten seedlings were transferred to each plastic container. The growth system in this experiment is shown in Fig. 1. Each plant was put on a nylon net that floated on the culture solution. Plants were grown in a phytotron (System Biotron, Nippon Medical and Chemical Instrument) at a temperature of about 23.5°C with air bubbling through it. It was irradiated with 5000 lux of light for 14 h a day. The elongation rate of the primary root was measured for 3 days.

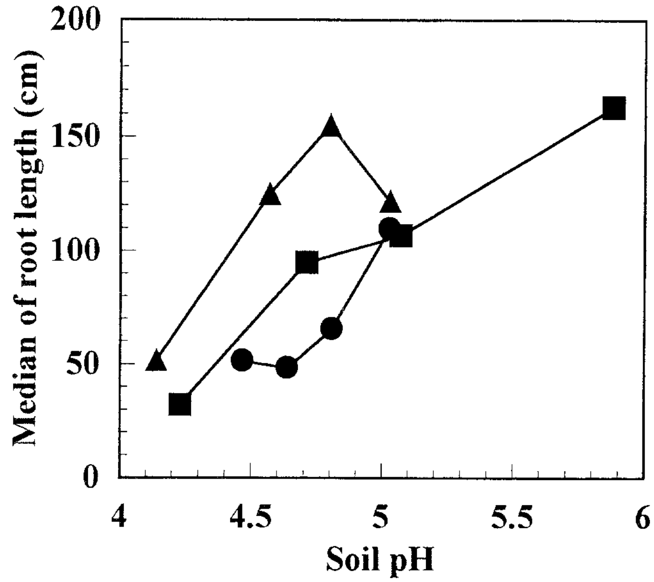

Fig. 2. Effect of a modified lignin on elongation of radish root in soil. *Squares*, controls; *circles*, aluminum (AL); *triangles*, aluminum + modified lignin (L8)

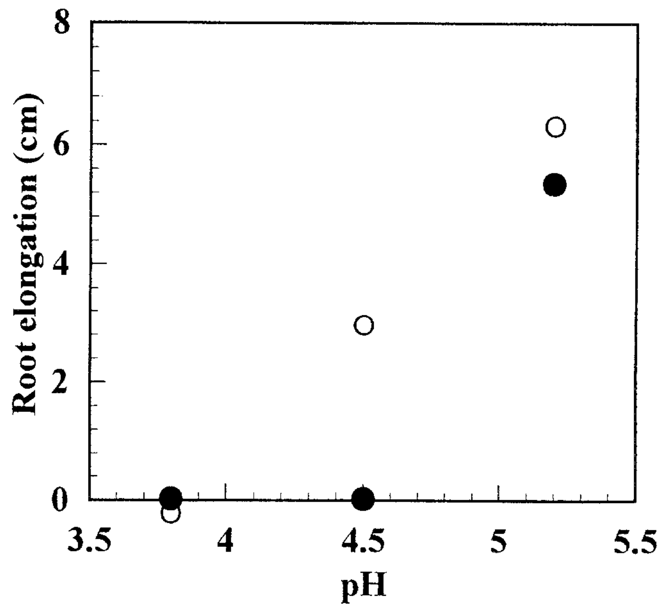

Fig. 3. Effect of pH on elongation of radish root. *Open circles*, controls; *filled circles*, AL 50ppm

Detection of AL adsorption to the root by hematoxylin staining

Four-day radish seedlings were kept in solutions containing 0.2mM $CaCl_2$, 5ppm AL, and 0–700ppm L4 for 6h. They were then washed with deionized water for 30min with air bubbling. These seedlings were then stained with a solution containing 0.2% hematoxilin and 0.02% KIO_3 for 30min. Excess hematoxylin was washed off by deionized water with air bubbling, and the root color was observed using a stereomicroscope.[14]

Results and discussion

Root growth in soil

The root length of radish grown in soil was measured by the line intersection method.[15] Although the pH of the soil solution was not constant throughout the growth test, plant growth was considered to be dependent mainly on the initial pH (data not shown). The median values of the plant growth versus the initial pH are plotted in Fig. 2. Because the pH effect was not remarkable around pH 4.7, it might be possible to compare root growth with and without addition of AL in this range. Aluminum ions suppresses radish root growth. When modified lignin was added to the soil containing AL, however, radish root growth was apparently increased. In other words, AL toxicity was reduced by a modified lignin in the pH range 4.5–4.8.

At pH 5.0 there was no obvious difference among these three conditions. Aluminum toxicity was not observed regardless of the addition of a modified lignin. This may indicate that AL was hydrolyzed and polymerized and was insoluble in water. Another important point is the difference in toxicity between free AL and the AL–lignin complex, which is discussed later.

Some of the AL may be dissolved from the soil into solution under acidic conditions. In practice, slight AL elution was detected in the control solution with lower pH (data not shown), which may be one of the reasons for the decreased root length at pH 4.2 even without addition of AL. This is another difficulty when trying to control the AL concentration precisely in soil.

Root growth in solution

The elongation rate of primary root grown in a culture solution was measured. Ten samples were used for each condition, and the results were evaluated statistically after removing extreme values using Dixon's Q method.[16]

The effect of pH on root elongation is shown in Fig. 3. A culture solution of low pH showed clear inhibition of root elongation, and root elongation was not observed at pH 3.8 even in a culture solution without AL. This finding indicates that radish cannot grow at such a low pH. However, root elongation in the presence of AL was as good at pH 5.2 as that in the control culture. In this case AL was polymerized and become insoluble in the culture solution owing to the high pH, the cutoff probably being pH 5. These results were in good agreement with the results for the soil shown in Fig. 2.

When the AL concentration in the solution was changed from 0ppm to 10ppm at pH 4.5, radish root elongation changed markedly (Fig. 4). The elongation was fatally inhibited with 5ppm AL and was completely inhibited in the presence of 10ppm AL. In this case, AL adsorption to root and inhibition of root elongation were thought to occur at the initial encountering with AL. It is interesting to note

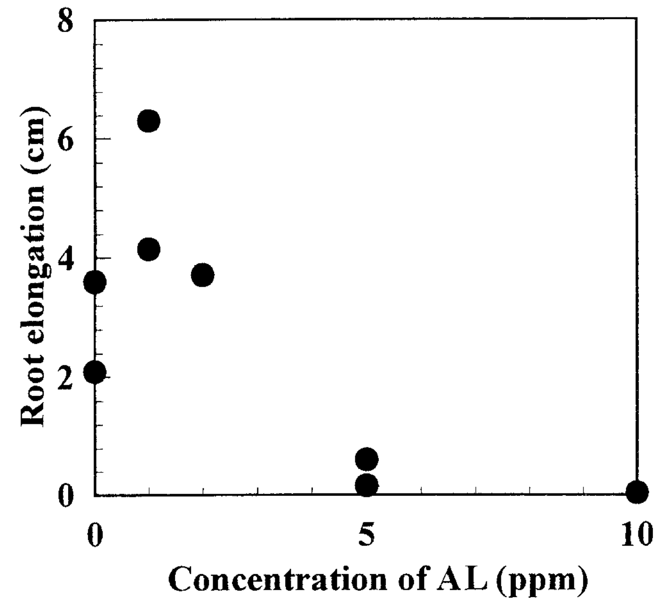

Fig. 4. Effect of AL concentration on elongation of radish root at pH 4.5

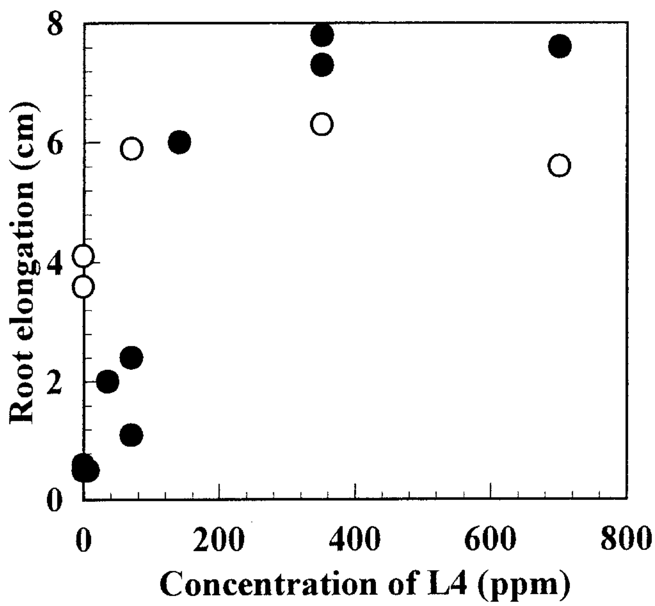

Fig. 5. Effect of a modified lignin (L4) on elongation of radish root at pH 4.5. *Open circles*, controls; *filled circles*, AL 5 ppm

that even a very low concentration of AL promotes root elongation.

As shown in Figs. 5 and 6, root elongation was promoted in the presence of kraft lignin and a modified lignin in a culture solution containing AL. A concentration of 140 ppm was needed for an alkaline oxygen-treated lignin (L4) to cancel the negative effect of 5 ppm AL. If the acidic groups, carboxyl groups, and phenolic hydroxyl groups were 0.6 eq in 200 g of L4, twice (molar) as much of the acidic groups was needed to cancel the negative effect of AL. In the case of kraft lignin (KL), about 2.5 times as much L4 was needed to attain a similar effect. Because muconic acid type

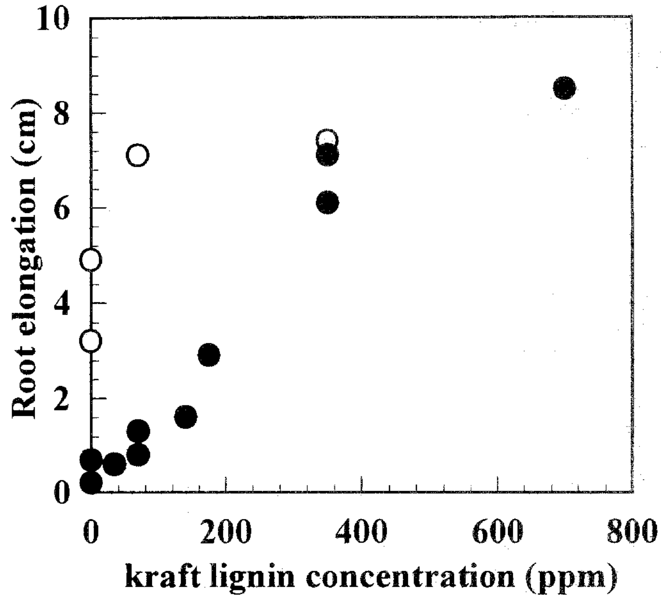

Fig. 6. Effect of kraft lignin on elongation of radish root at pH 4.5. *Open circles*, controls; *filled circles*, AL 5 ppm

structures were expected to be generated from phenolic structures in lignin by the ring-opening reaction during alkaline oxygen treatment, a larger amount of acidic groups in L4 than in KL is reasonable, but it may not be enough to explain the better ability of L4 compared to KL to remove the toxic effect of AL. The difference in the nature of the acidic groups in KL and L4 must be taken into account. As effective structures for the reduction of AL toxicity, catechol- and phenolic-type structures in the case of KL are expected, and muconic acid-type structures should be more important in the case of L4.

Effect of lignin on radish root elongation

In addition to promotion of elongation of radish root by lignins in the presence of AL, it is important to note that the promotional effect was observed even with lignin alone (Figs. 5, 6). At this time it is not clear why lignin effectively promotes radish root elongation. Further investigation is needed to elucidate this growth-promotion effect of lignin.

Hematoxylin staining

Although it is seen that lignin has a positive effect on reducing AL toxicity based on the results of this study, the effect of lignin alone, if any, could not distinguish clearly from the effect of the lignin–AL complex. To confirm that adsorption of AL to the root tip is disturbed in the presence of lignins, roots were treated with hematoxylin. Root tip grown in a culture without AL did was not stain, whereas the one grown in a culture with AL stained purple (darkly colored part of the root in Fig. 7), which indicates the presence of the hematoxylin–AL complex. It is important to note that root tip grown in a solution containing 350 ppm L4 and

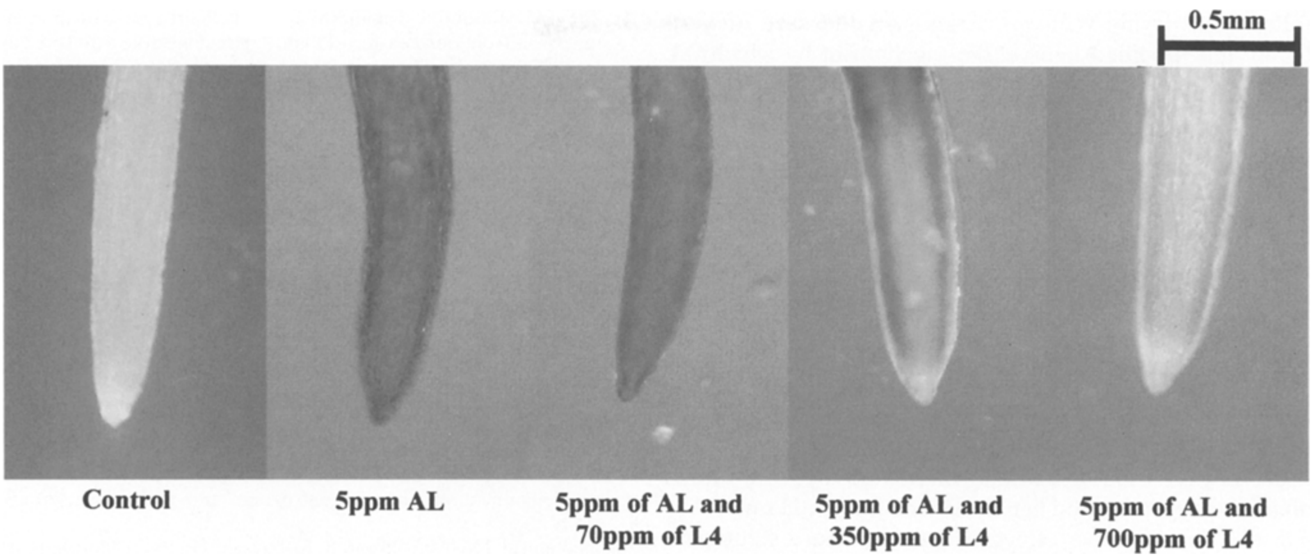

Fig. 7. Hematoxylin staining of radish root tip kept in solutions containing aluminum ions (*AL*) with or without addition of a modified kraft lignin

5 ppm AL was stained only slightly purple. This indicates that AL adsorption to the meristem of the root tip was effectively reduced by lignin.

Soluble AL and L4 in culture

The amounts of soluble AL and soluble lignin were measured after addition of L4, and the results are shown in Fig. 8. It is apparent that the amount of soluble Al was decreased with L4 up to 350 ppm, indicating precipitation of AL by the formation of aggregates between AL and L4. This did not occur when the L4 concentration was more than 350 ppm, at which condition a large amount of AL remained in the solution even after addition L4. When the L4 concentration was high, excessive carboxyl groups in the complex were free, making the aggregate water-soluble. It is interesting to note that although the soluble AL in the culture was at a high level when the L4 dosage was high, elongation of radish root was not obviously inhibited. This suggests that the reduction of AL toxicity to the plants was due to the complex formation between AL and the acidic groups in L4.

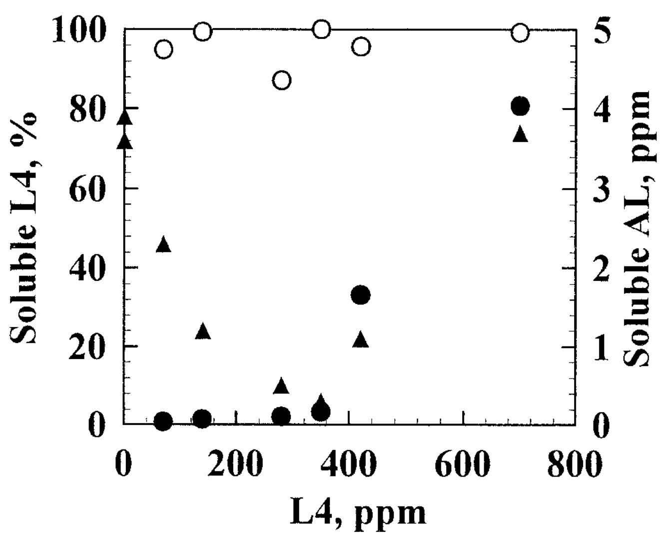

Fig. 8. Soluble L4 and aluminum ions (*AL*) at various L4 dosages. *Open circles*, soluble L4 in controls; *filled circles*, soluble L4 in presence of 5 ppm AL; *triangles*, soluble AL at 5 ppm

Why modified lignin effectively reduced AL toxicity

As discussed before, radish was grown normally with no toxic effect of AL after lignin addition at the same pH. Two explanations for the reduced AL toxicity were considered. (1) The growth promotion by lignin is more significant than the degree of growth inhibition by AL. Because it is not clear why lignin promotes root elongation, it is difficult to discuss this explanation at the moment. This explanation may not be the main one, however, as AL adsorption to the root surface is almost completely inhibited by addition of 350 ppm L4 (Fig. 7). (2) Aluminum toxicity is reduced by lignin via two possible pathways: AL forms a water-insoluble aggregate with lignin, or the AL–lignin aggregate is still soluble but cannot affect the plant. The former was shown for 140 ppm L4 to 5 ppm AL (Figs. 5, 8). The latter was apparent from the reduced AL toxicity with the higher L4 concentration (700 ppm) despite the presence of soluble AL (Figs. 5, 8).

Humic substance originating from dead plants and animals via degradation by microorganisms is the organic matter in soil that has a large number of functional groups (e.g., carboxyl, carbonyl, and phenolic hydroxyl groups). Humic substances form stable complexes with polycations in soil, and the complexes thus formed are not soluble in solution. It is reported that the stability constant of the complex follows the Irving-Williams series.[17] In the case of kraft lignin and modified lignins, the mechanism by which AL toxicity is reduced might be similar to that exhibited by soil organic, humic substances.

Conclusions

In the search for a soil-conditioning agent for acidic soil containing excess AL, kraft lignin was modified by alkaline oxygen treatment. The growth of radish root in solution and in soil containing AL with or without the addition of these lignins under controlled pH conditions was examined. We concluded the following. (1) Growth inhibition of radish roots in soil by AL can be eliminated by addition of alkaline-oxygen treated lignins to sand in the pH range 4.5–4.8. (2) The negative effect of AL in culture solution is decreased by addition of a modified lignin at pH 4.5. A twice-molar amount of alkaline oxygen treated lignins to AL, in terms of the acid groups, is necessary to cancel the toxic effect of AL. In the case of purified kraft lignin, a similar effect is obtained at a lignin concentration of about 2.5 times that of the modified lignin. (3) Although the soluble AL in the culture solution is at an extremely high level, when the dose of a modified lignin is high enough radish root elongation is not obviously inhibited. This suggests that AL toxicity to plants can be reduced by formation of a complex between AL and acidic groups in the modified lignin. (4) Aluminum accumulation at root tips growing in a solution culture containing AL is observed by hematoxylin staining. Accumulation at the root tip, however, disappears after addition of a large amount of alkaline oxygen treated kraft lignin. It is concluded that this modified lignin can inhibit adsorption of AL to the root tip, thereby avoiding AL toxicity to the radish root. The efficacy of the modified lignin to suppress AL toxicity is thought to depend on the lignin structure.

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