ZINC ADSORPTION BY STERILIZED AND NON-STERILIZED SOIL IN THE PRESENCE OF CITRATE AND CATECHOL

P. Chairidchai and G. S. P. Ritchie

ABSTRACT: The effect of organic ligands on zinc (Zn) adsorption in the rhizosphere may be influenced by microbial activity depleting the concentration of the ligand over time. Zinc adsorption by sterile and non-sterile lateritic soil was measured by shaking the soils with Zn solutions at a soil-liquid ratio of 1:5 for five periods of time ranging from 17-480 h. The concentrations of Zn and the ligands were determined after the selected times. The speciation of Zn was then estimated.

Changes in Zn adsorption with time in the presence of organic ligands were due to changes in pH and Zn complexation associated with the addition and breakdown of the organic ligands and with the technique of sterilization. Zinc adsorption was higher in non-sterile than in sterile soil. Adsorption in the presence of citrate was lower than in its absence except in non-sterile soil after 72 h. The adsorption in the presence of catechol was similar to that in its absence.

INTRODUCTION

The adsorption of Zn has been demonstrated to be influenced by the presence of organic ligands (1,2). However, the effect of organic ligands on the adsorption of Zn by soil in rhizosphere may be altered due to microbial activity. In the rhizosphere, the population of micro-organisms can be 10 times greater than in the bulk soil (3,4), and therefore, their activity in this zone is believed to be high (5). Soil micro-organisms can often use organic ligands as their substrates (6) which may cause a depletion of the ligand concentration in the rhizosphere. However, the rhizosphere is a dynamic system where the production of organic root exudates is occurring frequently. The concentration of organic ligands at any one time can be quite variable due to the rate of their replacement which depends on factors such as plant age, soil temperature, and moisture and nutrient availability (7,8).

Our objective was to study the effect of organic ligands on Zn adsorption by non-sterile and sterile soils after various periods of time. Citrate and catechol were chosen because Zn adsorption has been found to be smaller in the presence of citrate than in its absence whereas catechol did not have any effect on adsorption (2). Unlike the rhizosphere, the system used in this experiment was much simpler in that there was no replacement of ligands taking place. Therefore, the effect of organic ligands varied with time.

MATERIALS AND METHODS

The influence of soil micro-organisms on the effect of organic ligands on zinc adsorption by a lateritic soil was studied by measuring adsorption in sterilized and non-sterilized soil (Northcote classification: Dy 5.51, Soil Taxonomy: Entisol) after 17 to 480 h. The soil was collected from Bakers Hill, Western Australia and contained 0.004 µmol/g of DTPA-extractable Zn, 2.1% organic C, and had a cation exchange capacity of 6.9 $cmol_c$/kg.

A solution of 0.003M KCl containing 2.5 µmol/g $Zn(NO_3)_2$, and 0 or 1 mM of citric acid or catechol was sterilized by filtration (<0.22 µm). Twenty milliliters of the solutions were then shaken with duplicate, 4 g subsamples of sterile or non-sterile soil for 17, 72, 144, 240, and 480 h. The sterile soil was prepared by autoclaving (121°C, and 400 KPa, for 20 min twice, 24 h apart). After the selected times, the soil mixture was centrifuged (1000 g force), and then filtered (<0.45 µm). The pH of the filtrates were measured. Microbial counting (9) confirmed that sterilization was effective. The filtrates were analyzed for Zn (atomic absorption spectrometry), catechol (10), and citrate (11).

Zinc speciation was estimated by a chemical equilibrium program TITRATOR (12). Ionic strengths of solutions were calculated by the program to compensate for the contribution of components other than KCl. In all cases, calculated values of ionic strength were lower than 0.004. The species considered were the free Zn ion (Zn^{2+}), the hydrolysed Zn ($ZnOH^+$), and Zn citrate ($Zncit^-$) or Zn catechol complex (Zncat). The values for the log K used in the calculation were from Lindsay (13) and Martell and Smith (14).

The data were analyzed by linear regression. Standard errors were estimated and are shown as error bars in the figures if the standard error was greater than the diameter of the data point.

RESULTS

Zinc Adsorption: Zinc adsorption in all treatments increased with time (Fig. 1). For sterile soils, the maximum adsorption was reached after 240 h (Fig. 1a) whereas the increase in the adsorption by non-sterile soil between 17 and 480 h was never greater than 6% (Fig. 1b).

The effect of the ligands on Zn adsorption depended on the ligand type. Catechol did not affect Zn adsorption either in sterile (Fig. 1a) or non-sterile soil (Fig. 1b) . On the other hand, the effect of citrate varied with the sterilization. In sterile soil, the presence of citrate decreased Zn adsorption at all times (Fig. 1a). In non-sterile soil, a marked decrease in Zn adsorption due to the presence of citrate occurred after 17 h, but the decrease became smaller and eventually disappeared at later times (Fig. 1b).

Sterilization also affected Zn adsorption. At constant time, adsorption was greater in a non-sterile than a sterile soil (Fig. 1). The sterilization effect was greatest in the presence of citrate (Fig. 1). In the absence of a ligand or in the presence of catechol, the difference was less than 1%.

In the presence of organic ligands, Zn adsorption decreased linearly ($r^2 > 0.99$) with the increasing concentration of $Zncit^-$ (Fig. 2a), exponentially with Zncat (Fig. 2b) and more than 99% of its variation could be explained by the combined effect of both complexed species and $ZnOH^+$ in a single equation. Adsorption also increased with pH (Fig. 3).

Zinc in Solution: Zinc concentration in soil solution decreased with time (Fig. 4) . The initial decrease (from 17 - 48 h) was greatest in the presence of citrate, the effect being greater in the non-sterile soil than in the sterile soil. Zn concentration in solution of the non-sterile soil at 480 h was only 18.9% of that at 17 h in the presence of citrate, whereas it was 44.6% in the sterile soil.

The concentration of each Zn species in solution varied with time and sterilization methods (Fig. 5) Both $Zncit^-$ and Zncat decreased with time in solutions from sterile and non-sterile soils (Fig. 5a and b). However, the decrease of both species was greater in the non-sterile soil. Zinc (Zn^{2+}) in all treatments, except in the presence of citrate in the non-sterile soil, also decreased with time

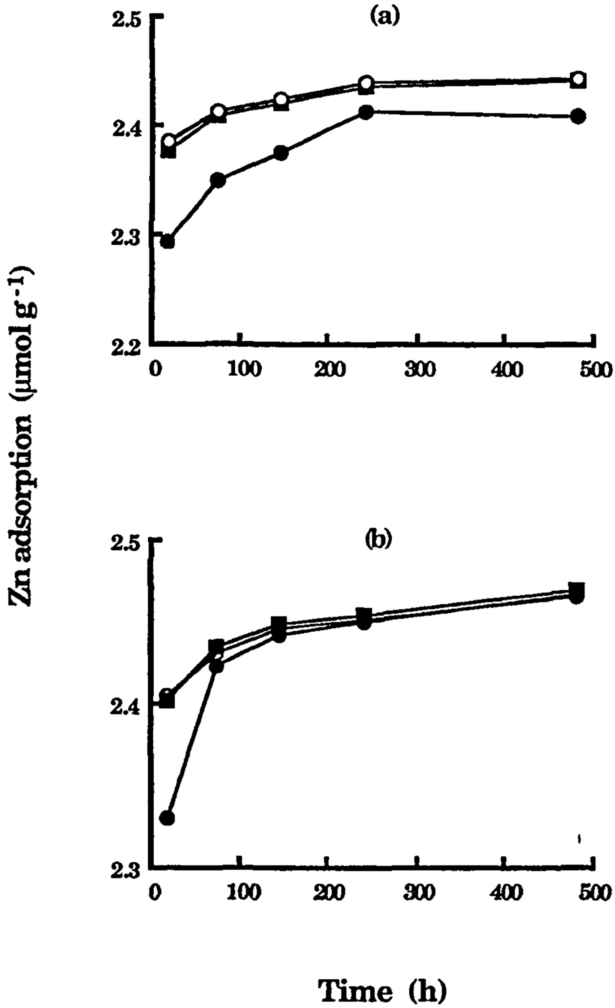

FIGURE 1. The relationship between reaction time and Zn adsorption by a lateritic soil in the absence (○) or in the presence of citrate (●) or catechol (■) by (a) sterile and (b) non-sterile soils.

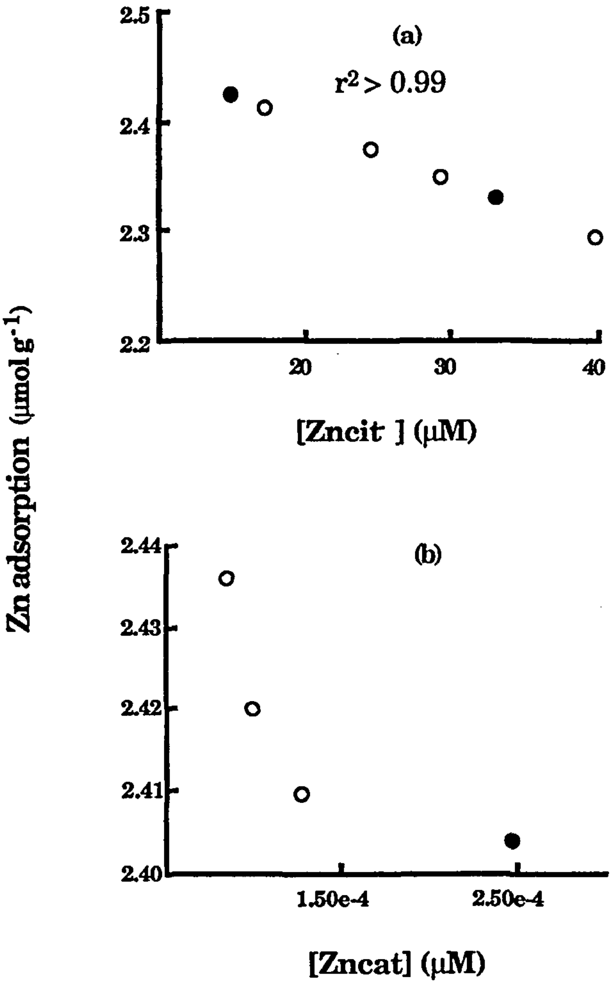

FIGURE 2. The relationship between the concentration of (a) $Zncit^-$ and (b) Zncat on Zn adsorption by sterile (○) and non-sterile soils (●).

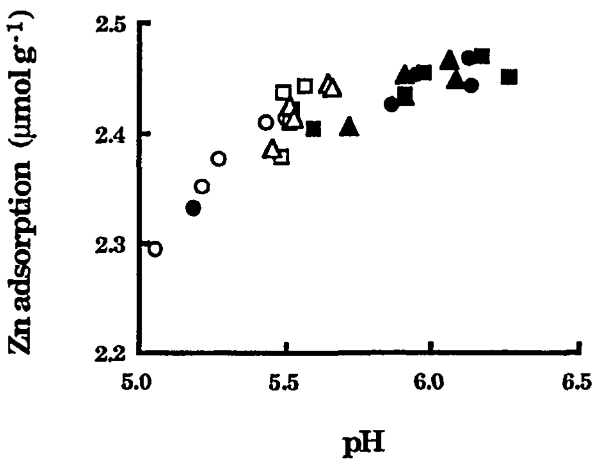

FIGURE 3. The relationship between pH and Zn adsorption by sterile soil (open symbols) and non-sterile soils (closed symbols) in the absence (Δ, ▲) or the presence of citrate (○, ●) and catechol (□, ■).

(Fig. 6a and b). In the latter case, there was an increase between 72 and 144 h (Fig. 6b). $ZnOH^+$ in the sterile soils generally decreased with time (Fig. 6c). In non-sterile soils, $ZnOH^+$ increased from 17 to 144 h, decreased up to 240 h and remained unchanged up to 480 h (Fig. 6d). The increase between 17 and 144 h was more marked in the presence of citrate than catechol which was more marked than in the absence of a ligand (Fig. 6d).

Ligand Concentrations: Sterilization also affected the ligand concentrations in the soil solutions. In the non-sterile treatments, the concentration of citrate decreased markedly from 631 μM at 17 h to zero at 144 h, whereas the concentration changed only slightly in the sterile soil (Fig. 7a). The concentration of catechol decreased from 789 μM at 17 h to zero within 72 h when the soil was not sterilized. After sterilization, however, catechol concentrations remained higher than 534 μM until 240 h (Fig. 7b).

The effect of pH on the concentrations of the organic ligands depends on the type of ligand. Citrate concentration decreased with increasing pH (Fig. 8a) whereas the concentration of catechol was independent of pH (Fig. 8b).

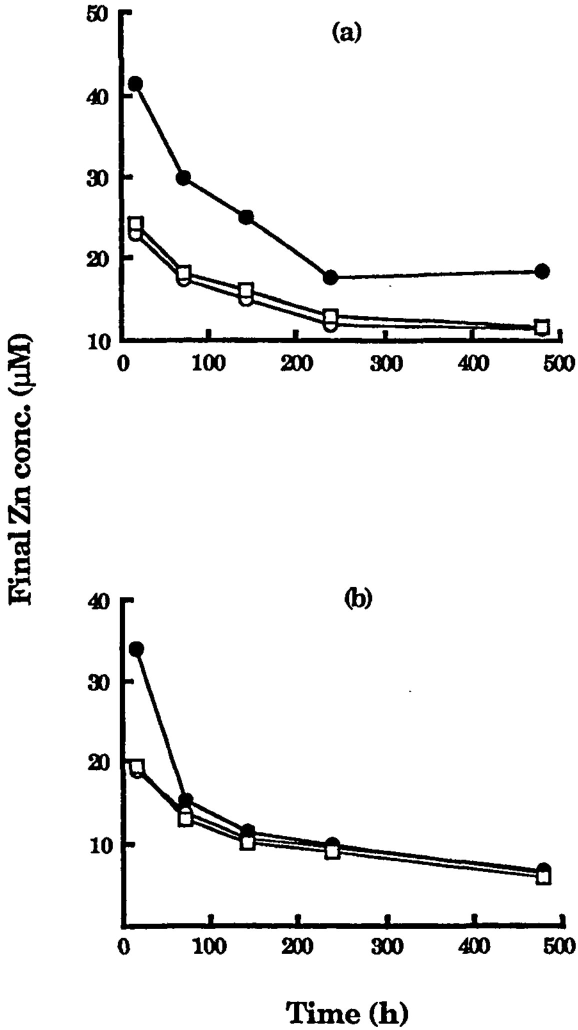

FIGURE 4. The relationship between reaction time and the concentration of Zn in solution of (a) sterile and (b) non-sterile soils in the absence (○) or presence of citrate (●) or catechol (■).

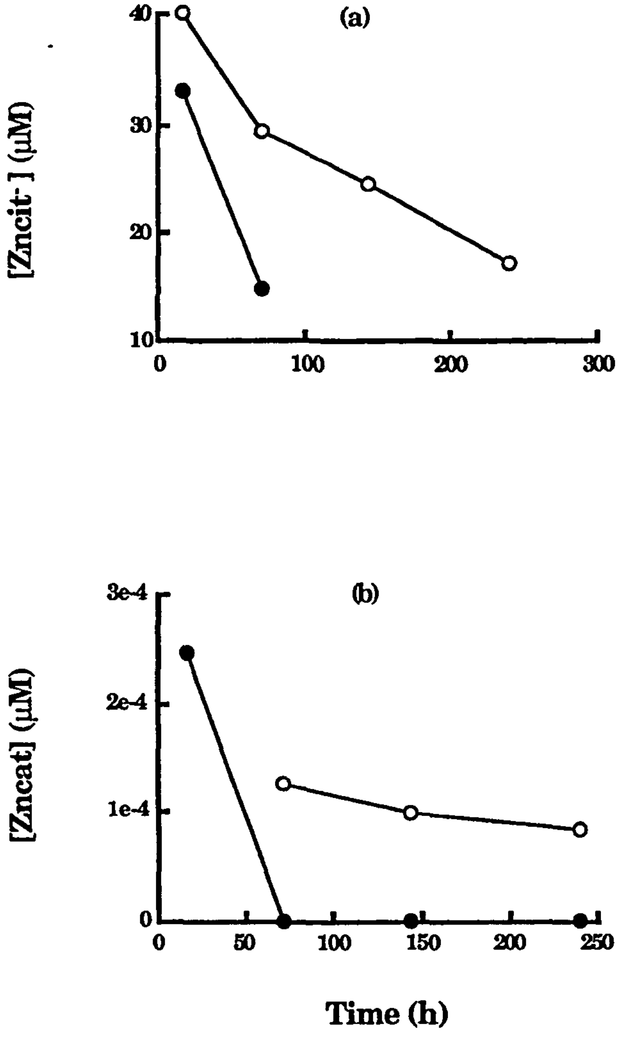

FIGURE 5. The relationship between reaction time and the concentration of (a) $Zncit^-$ and (b) Zncat in the sterile (○) and non-sterile soils (●).

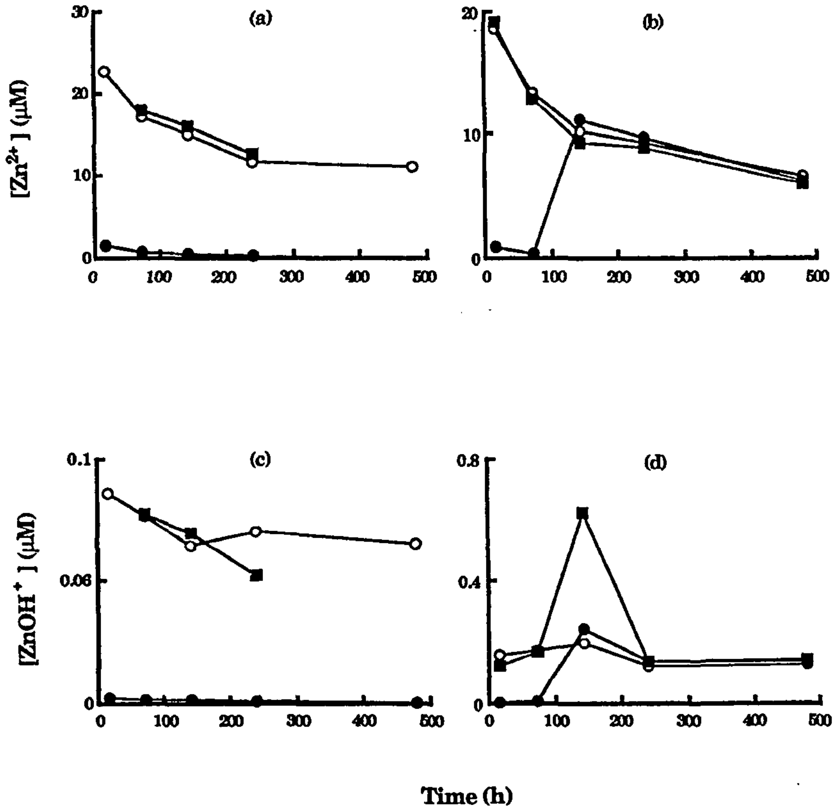

FIGURE 6. The relationship between reaction time and the concentration of Zn^{2+} (a,b) and $ZnOH^{+}$ (c,d) in the absence (○) or presence of citrate (●) or catechol (■) in (a,c) sterile and (b,d) non-sterile soils.

pH: At a constant time, sterilization decreased soil pH in the absence of organic ligands. The pH of soil solution increased with time (Fig. 9). In the presence of citrate and catechol, the pH of non-sterile soil increased more rapidly than those in the other treatments in the first 144 h, and the increase up to this time was as high as 0.9 of a pH unit in the presence of citrate and about 0.5 unit in the presence of catechol. However, the pHs of these soils dropped 0.2-0.3 units from 144 to 240 h before increasing again. The pH of the sterile soils increased only marginally within the studied length of time.

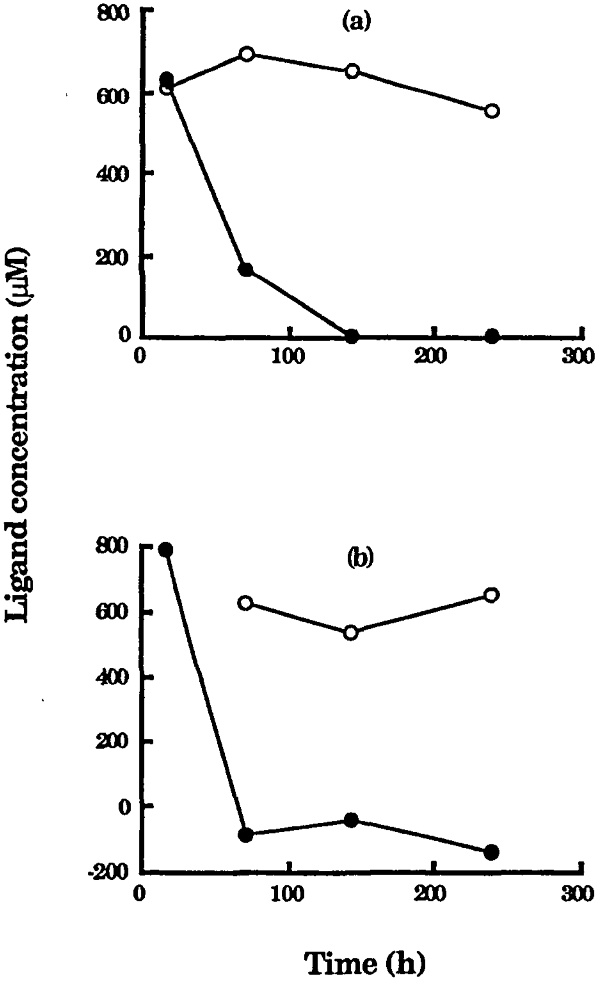

FIGURE 7. The relationship between reaction time and the concentration of (a) citrate and (b) catechol in sterile (○) and non-sterile soils (●).

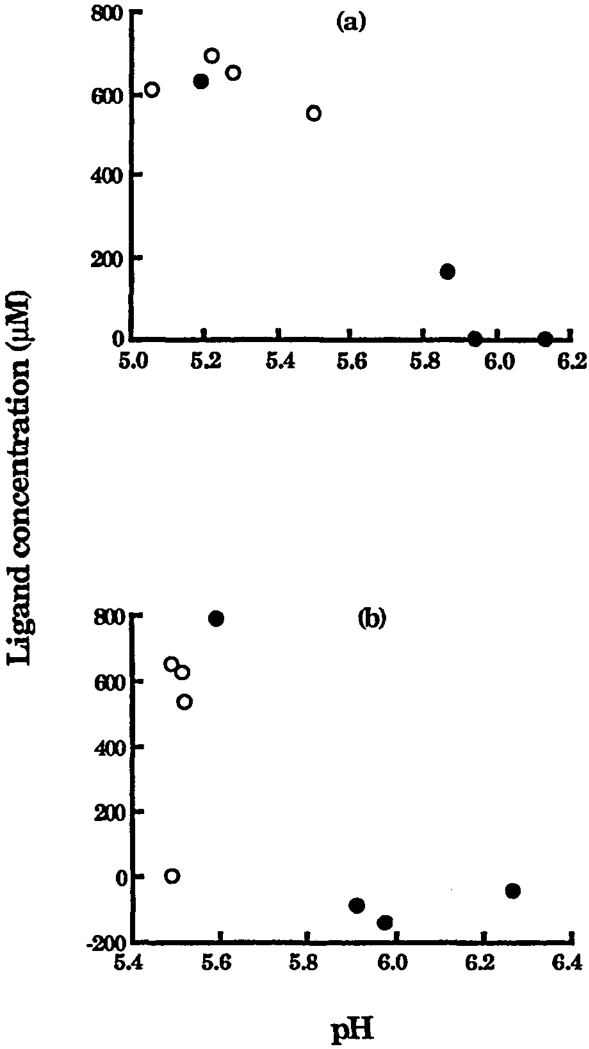

FIGURE 8. The relationship between pH and the concentration of (a) citrate and (b) catechol in sterile (○) and non-sterile soils (●).

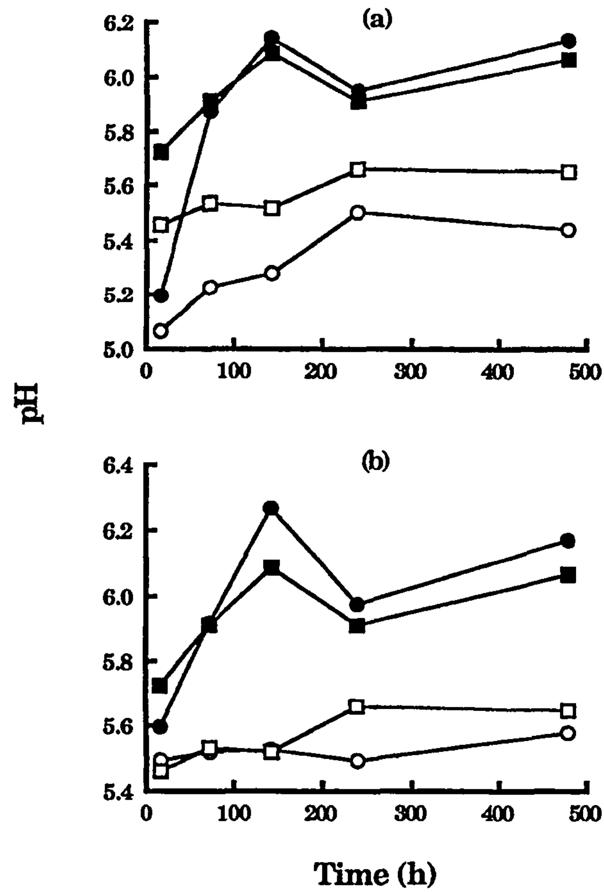

FIGURE 9. The relationship between reaction time and the solution pH in the absence (square symbols) or presence (round symbols) of (a) citrate and (b) catechol in sterile (open symbols) and non-sterile soils (closed symbols).

DISCUSSION

Changes in Zn adsorption with time in the presence of organic ligands were related to changes in pH and Zn complexation associated with the addition and breakdown of the organic ligands and the technique of sterilization.

Zinc adsorption was demonstrated to be greater in the non-sterile than in the sterile soil, particularly in the presence of citrate. The greater adsorption was due to the higher pH and the lower ligand concentrations in the non-sterile soils than in the sterile soils. The higher solution pH of the non-sterile soils was partly due to the activities of the soil micro-organisms that were present and partly due to the technique of sterilization.

Sterilization decreased pH at any one time interval and prevented microbial decomposition of the organic ligand. Both these factors decreased adsorption if a Zn ligand complex was formed.

As time of adsorption increased, Zn adsorption by unsterile soil increased due to the breakdown of organic ligands which had initially prevented Zn complexation, and due to an increase in pH due to the addition and breakdown of the ligands. In sterilized soil, Zn adsorption also increased with time due to changes in pH which were related to the addition of citrate but not to its decomposition. The latter mechanism had the least effect on adsorption. The increase in adsorption with time could also have been partly due to Zn ingestion by micro-organisms in non-sterile soils. This could explain the small increase ($< 6\%$) in Zn absorption observed between 17 and 240 h.

The effect of the reaction time on the pH of the soil solution may have been due directly or indirectly to the activities of the soil micro-organisms. The larger increase in pH of the non-sterile soil than that of the sterile soil in the absence of a ligand may indicate the direct effect of the microbial activity, whereas the larger increase in non-sterile soil than in the sterile soil in the presence of citrate may indicate the indirect effect of the microbial activity.

A comparison of the pH of non-sterile soil in the presence and absence of citrate indicated the breakdown of the ligand included the production of OH^- or consumption of H^+. The change in pH due to the addition of the ligand in sterile soil was only observed for citrate and presumably was a result of pH buffering by the soil neutralizing dissociated H^+ from the citric acid. Catechol would be completely associated at the initial pH of the soil, and hence, would not be expected to decrease pH.

The organic ligands decreased Zn adsorption in a similar manner as found in earlier work (2). In the presence of citrate, the charge on the complex species is an important factor in determining the adsorption of Zn. The pH of solution in this study were all higher than p.z.s.e. of the soil [p.z.s.e. = pH 3.9; (2)]. The adsorption of Zn, therefore, decreased with increasing concentration of $Zncit^-$ because the negatively charged Zn species was not adsorbed onto the negatively charged surfaces (16). In the presence of catechol, the adsorption of Zn was similar to that in its absence because catechol complexed only slightly with Zn, and hence, there was no effect of catechol on Zn adsorption (2). However, most of the variation in Zn adsorption in this study could also be explained by the combined effect of the complexed Zn species plus $ZnOH^+$.

The results of this work indicated that the effect of organic ligands is only transitory (17 - 48 h) if the organic ligands are broken down by microorganisms. In the rhizosphere, however, organic ligands such as citrate are being continually produced either by exudation from plants (16), or by microbial breakdown of more complex organic material (17,18). Therefore in the dynamic environment of the rhizosphere, organic ligands may still have an important influence on Zn concentration in solution.

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