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MODELING THE REACTIONS OF 1-NAPHTHYLAMINE AND 4-METHYLANILINE WITH HUMIC ACIDS: SPECTROSCOPIC INVESTIGATIONS OF THE COVALENT LINKAGES

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Abstract—The covalent binding of two aromatic amines, 1-naphthylamine and 4-methylaniline, to substituted quinones in aqueous solutions has been studied as a model for the covalent binding of amines to humic acids. An imine compound was the only type of product identified for the reactions between 1-naphthylamine and selected quinones. Reactions of 4-methylaniline with the same quinones gave a 1,4-addition product in addition to the imine compound, although only the imine product was formed when the quinone had bulky substituents at the 2- and 6- positions. These results provide additional insight into the reactions of aromatic amines with humic acid quinones.

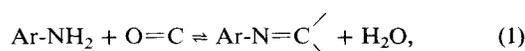
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INTRODUCTION

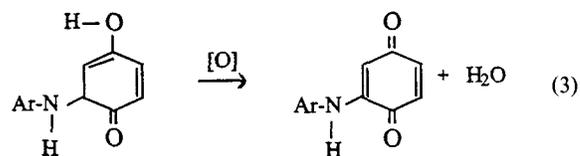
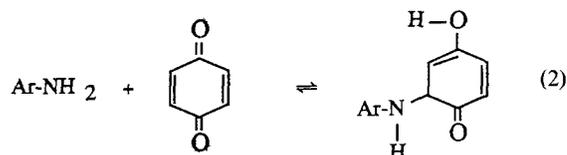
Aromatic amines such as benzidine, 1-naphthylamine, and 4-methylaniline have been identified as potential carcinogens [1,2]. These compounds are used in the manufacture of dyes, paints, textiles, and other related products. Like most pollutants, toxic aromatic amines may enter the soil through various means, including accidental spills and unprotected waste disposal. Because they are nonvolatile and tend to persist in soil, their fate in soil has been the focus of a number of investigations [3-8].

When aromatic amines are applied to soils, they are retained mainly by the humic fraction of soil organic matter and are not readily degraded by indigenous microorganisms [3-5, 7, 9, 10]. Humic acids as a model for the humic fraction are complex but are known to contain substituted quinonoid groups [11,12]. Studies have shown strong similarities between humic acid-aniline and quinone-aniline products [5,10]. Therefore, quinones are generally used as models for humic acids in mechanistic studies of the reactions of aromatic amines with humic acids.

Anilines are a class of simple aromatic amines that have been extensively investigated. Two mechanisms are postulated for their reactions with quinones [5]: a phase I reaction, which consists of initial rapid and reversible imine formation (Eqn. 1)



and a phase II reaction, which consists of a slow 1,4-nucleophilic addition of the amino group onto the quinone ring (Eqns. 2 and 3)



For the anilines, the reverse of the phase I reaction is believed to be very rapid in aqueous solutions, so that the product is not readily isolated. The 1,4-addition product is quite stable, however. A pertinent question is whether all the other aromatic amines behave similarly toward the quinones. In an investigation of the reactions of benzidine (a complex aromatic amine) with quinones, the imine product was found to form to an appreciable extent and to be stable in aqueous solutions [13]. This suggests that major differences can exist in the reactions of different aromatic amines with quinones and, thus, the need for additional studies into the behavior of various toxic amines.

Two stages are involved in studies of a reaction mechanism: reaction-product identification and reaction kinetics. The focus of this paper is product identification. The covalent linkages formed by the respective reactions of 1-naphthylamine (1-NPA) and 4-methylaniline (4-MA) with 2,6-dimethyl-*p*-benzoquinone (DMBQ) and 2,6-di-*tert*-butyl-*p*-benzoquinone (DTBBQ) have been spectroscopically investigated and presented here. The structures of the initial compounds are given in Figure 1.

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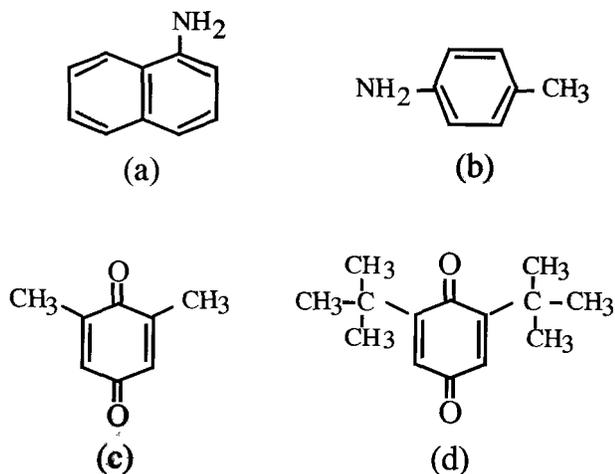


Fig. 1. Structures of the aromatic amines and quinones employed in this study: (a) 1-naphthylamine (1-NPA); (b) 4-methylaniline (4-MA); (c) 2,6-dimethyl-*p*-benzoquinone (DMBQ); (d) 2,6-di-*tert*-butyl-*p*-benzoquinone (DTBBQ).

MATERIALS AND METHODS

The quinones and amines were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. Reactants were mixed at 10 mmol concentration in 10-ml volumes at neutral pH and room temperature (24–26°C) in 250-ml stoppered Erlenmeyer flasks, covered with aluminum foil, and allowed to react for at least 7 d with continuous stirring by means of a magnetic stirrer. Products were filtered, washed with a copious amount of water, and separated sequentially by traditional liquid chromatography and HPLC. For the traditional separations a glass column (2.5 cm diameter by 60 cm long) was packed with Florisil (60–100 mesh), and elution was achieved with a 1:10 CH₃CN:CHCl₃ mixture. The HPLC runs were performed using a Waters (Mississauga, Ontario) model 510 pump, model 680 controller, model 484 detector, model 730 data module, and a C18 radial compression column. The mobile phase consisted of 40:60% CH₃OH:CH₃CN solution. Mass spectral data were obtained on a ZAB-EQ low-resolution MS by electron impact ionization, and proton nuclear magnetic resonance (NMR) data were obtained on a Jeol (Akihima, Japan) model FX90Q spectrometer.

RESULTS

The products formed during each reaction settled out of the aqueous reaction mixture, implying low solubility in water. The reactions were slow in all cases with <70% of the starting reagents (amines and quinones) consumed after 14 d, except for 4-MA, which reacted more rapidly with DMBQ. Characterization of the respective products is given as follows:

Products between 1-NPA and DMBQ

Only one product (reddish in color) was isolated from this reaction. Mass spectral analysis of the product (Fig. 2a) gave a molecular ion peak at 261 mass units, which corresponds to the calculated molecular mass for the structure proposed

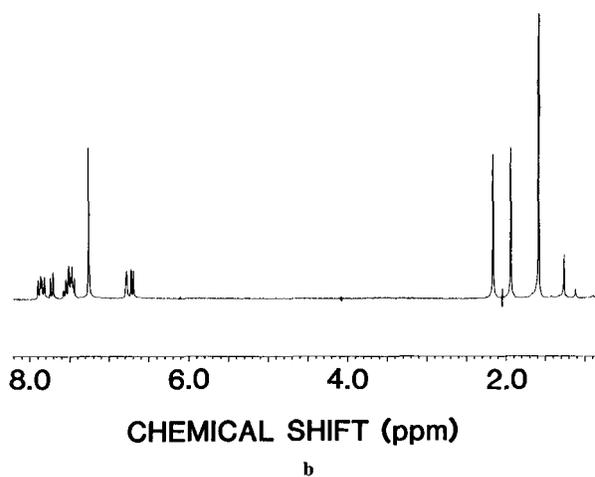
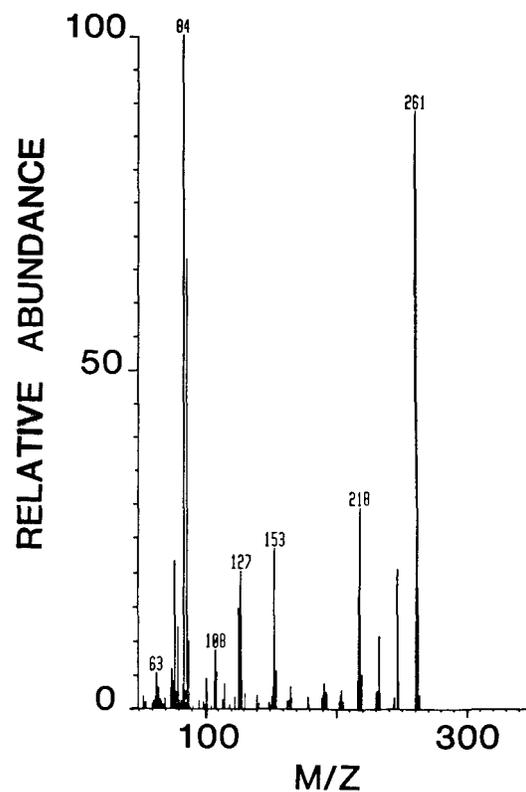


Fig. 2. (a) Mass spectral and (b) NMR data for the compound formed during the reaction between 1-NPA and DMBQ.

in Figure 3a (1-NPA-DMBQ). This is an imine compound, for which the structure can be further confirmed by using the NMR spectrum (Fig. 2b): The two unequivalent methyl groups on the quinone ring give rise to the peaks at 1.95 and 2.15 ppm. The protons on the benzene ring of the quinone are also unequivalent, giving rise to peaks at 6.80 and 7.25 ppm (although the 7.25-ppm peak is swamped in this case by the background peak inherent in the solvent [CDCl₃]). The rest

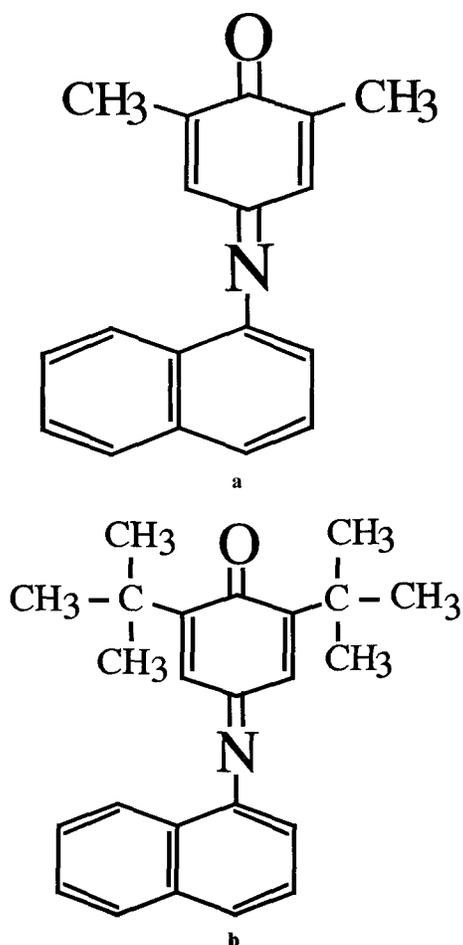


Fig. 3. Proposed structures for the compounds (a) 1-NPA-DMBQ formed in the reaction between 1-NPA and DMBQ, and (b) 1-NPA-DTBBQ formed in the reaction between 1-NPA and DTBBQ.

of the multiplets in the aromatic region of the NMR spectrum are typical of 1-naphthylamine aromatic protons. Noticeably absent are the typical NH or NH₂ absorption peaks, providing strong corroboration of the imine structure. The peaks at 1.25 and 1.60 ppm are inherent in the solvent.

Products between 1-NPA and DTBBQ

Again an imine compound was the only product obtained. The mass spectrum (with molecular ion peak at 345 mass units) and NMR results (Fig. 4) support the proposed structure of the compound (Fig. 3b). The NMR spectrum can be explained as for 1-NPA-DMBQ above. The protons on the tert butyl groups are more shielded than those of the methyl groups in 1-NPA-DMBQ; however, the peaks are shifted further into the aliphatic region at 1.15 and 1.40 ppm. Also, the peak at 7.25 ppm is well resolved from the solvent peak in this case.

Products between 4-MA and DMBQ

Two products, 4-MA-DMBQ1 (reddish in color) and 4-MA-DMBQ2 (deep purple) were isolated and identified by

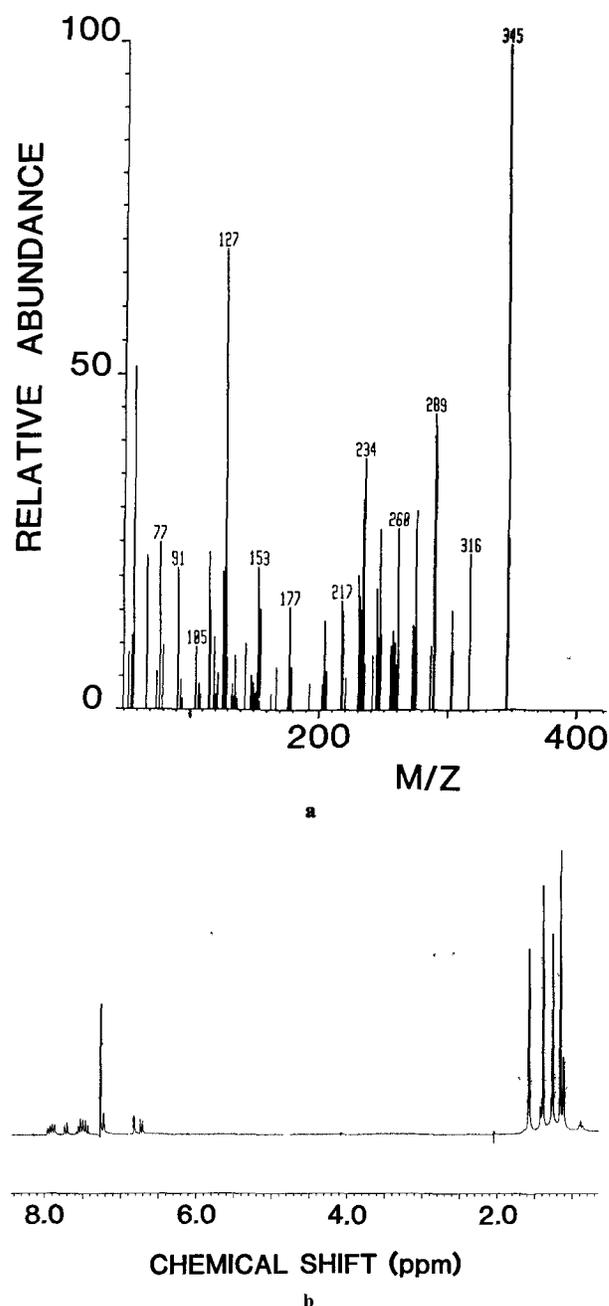


Fig. 4. (a) Mass spectral and (b) NMR data for the compound formed during the reaction between 1-NPA and DTBBQ.

mass spectral and NMR analysis as the imine (Fig. 5a) and 1,4-addition (Fig. 5b) products, respectively. The mass spectral data are given in Figure 6, with the molecular ion peaks at 225 (Fig. 6a) and 241 (Fig. 6b) mass units agreeing with the calculated molecular masses for 4-MA-DMBQ1 (Fig. 5a) and 4-MA-DMBQ2 (Fig. 5b), respectively. The mass ratio of the two products was approximately 4-MA-DMBQ1/4-MA-DMBQ2 = 1/3. Thus, the 1,4-addition product was formed predominantly.

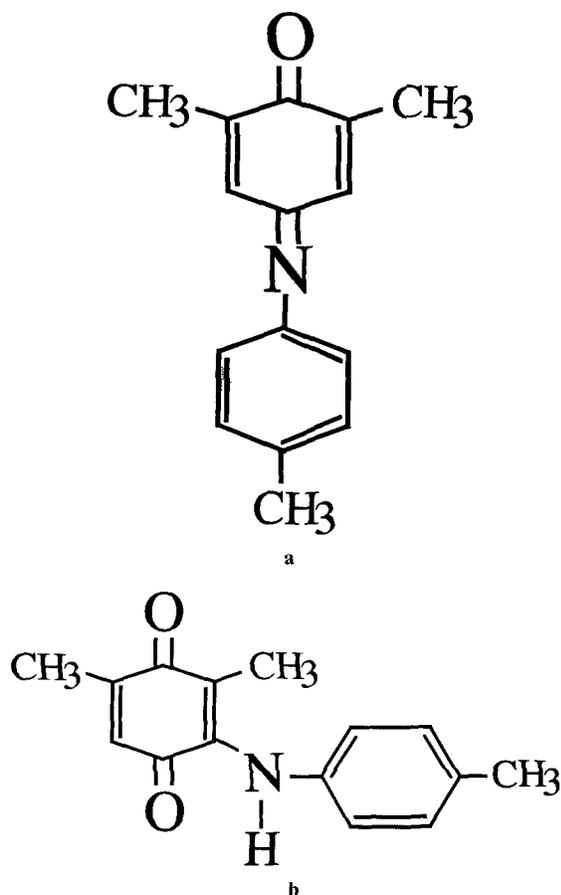


Fig. 5. Proposed structures of the compounds (a) 4-MA-DMBQ1 and (b) 4-MA-DMBQ2 formed in the reaction between 4-MA and DMBQ.

Products between 4-MA and DTBBQ

Only the imine compound 4-MA-DTBBQ was isolated following the reaction of 4-MA with DTBBQ. Its structure (Fig. 7) was deduced from spectroscopic data. The mass spectrum (Fig. 8) shows a molecular ion peak at 309 mass units, which is in agreement with the molecular mass for this structure.

DISCUSSION

Overall the above results indicate that the imine, and not the 1,4- addition compound, will always form during the reactions of aromatic amines with quinones. The imine is also the only product to be expected when the amine is as complex as 1-NPA. Absence of the 1,4- addition product in this case may be due to steric hindrance of the bulky 1-NPA, preventing effective orbital overlap at the quinone ring. The less bulky 4-MA forms both the imine and 1,4- addition products with quinones, but when large substituents are present on the quinone, only the imine product is obtained. This may also be due to steric hindrance from the substituents, preventing close approach of the amine.

In predicting the nature of the binding that should occur between aromatic amines and humic acids it should be noted

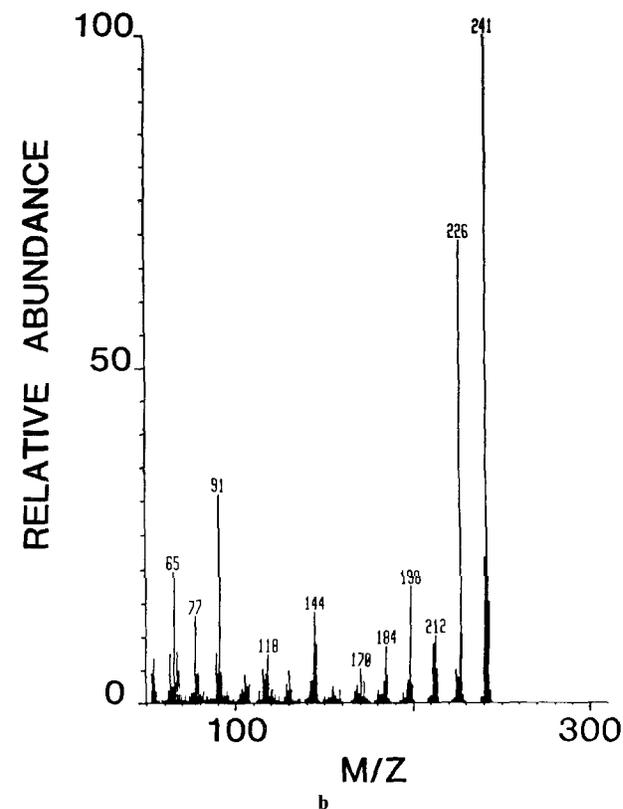
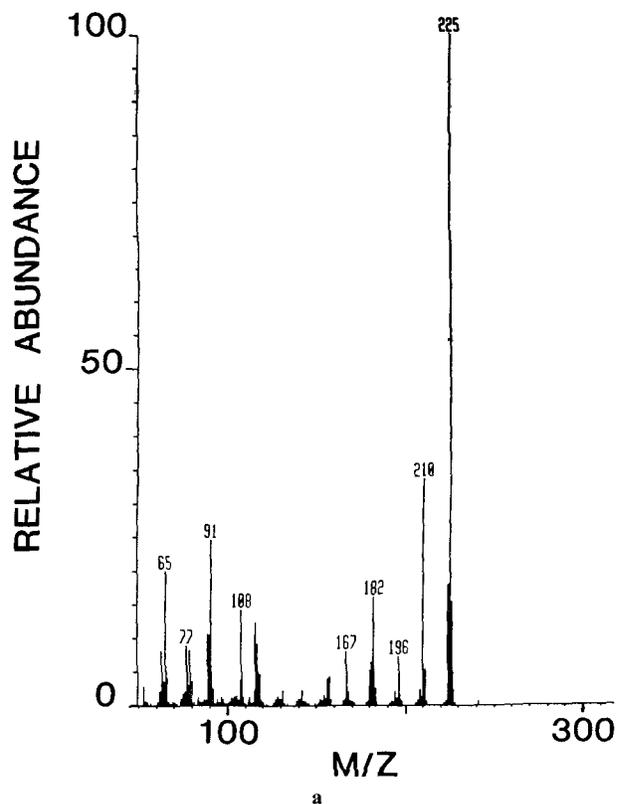


Fig. 6. Mass spectral data for the compounds (a) 4-MA-DMBQ1 and (b) 4-MA-DMBQ2 formed during the reaction between 4-MA and DMBQ2.

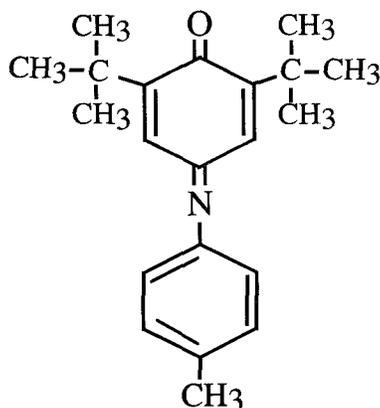


Fig 7 Proposed structure for the compound 4-MA-DTBBQ formed during the reaction between 4-MA and DTBBQ

that the proposed structures of humic acids suggest that their quinonoid groups are surrounded by bulky substituents [12]. The results of this paper suggest that binding of aromatic amines by humic acids should occur predominantly by imine formation. This finding contrasts previous suggestions that the 1,4-addition reaction would be the predominant mechanism for binding of aromatic amines by humic acids [5,10]. Only if the quinonoid groups of the humic acids do not have large substituents can binding by 1,4-addition occur.

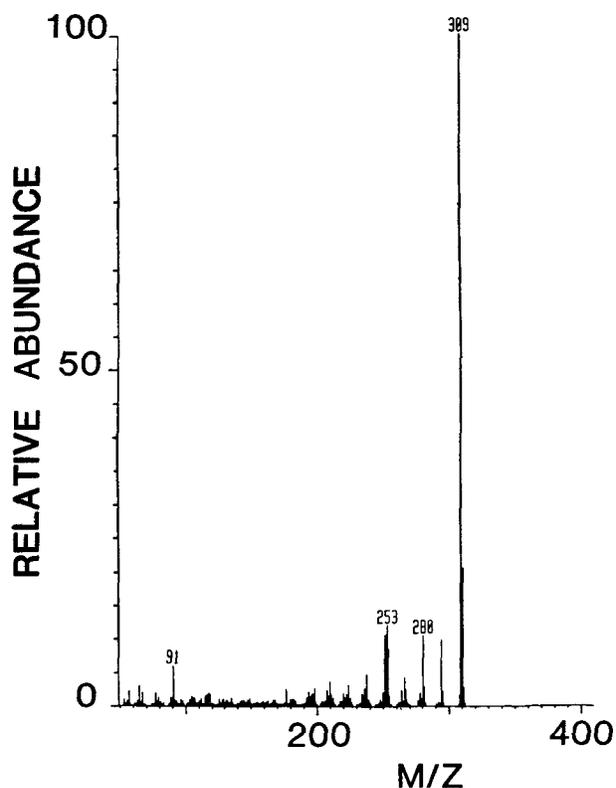


Fig 8 Mass spectrum of the compound 4-MA-DTBBQ formed during the reaction between 4-MA and DTBBQ

CONCLUSIONS

This study provides a pattern for the reactions of aromatic amines with complex quinones and, thus, more insight into the binding of aromatic amines by quinonoid groups in humic acids. Such reactions may provide a means of reducing the toxicity of aromatic amines in soils. Previous studies suggest that toxicity of xenobiotics may be reduced when they are incorporated into soil humus [14]. However, if the imine formation is predominant, then the binding reaction is reversible and therefore the aromatic amine would be biologically available in soil solution. Equilibration studies with benzidine show that the concentration in soil solution attains an equilibrium level after 2 d of incubation, thus suggesting a reversible reaction [15].

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