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on Fundamental and Applied Aspects of
Physical Chemistry

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and

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CONTENT

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OCHRATOXIN A AND ZEARALENONE ADSORPTION BY SURFACTANT MODIFIED ZEOLITE

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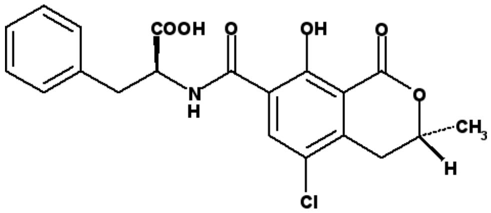
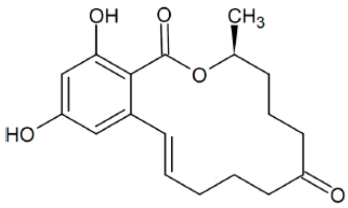
ABSTRACT
Cetylpyridinium chloride (CP) was used to modify the surface of the natural zeolite and adsorption of two mycotoxins, ochratoxin A (OCHRA) and zearalenone (ZEN) was investigated. The organozeolites were prepared with three different levels of CP (20, 50 and 100% of zeolite's external cation exchange capacity) and mycotoxin adsorption experiments were done at pH 3 and 7. Results showed that with increasing amounts of CP at the zeolitic surface, adsorption of OCHRA and ZEN increased, as well as with increasing amounts of solid phase in suspension. Adsorption of OCHRA was dependent of the form of OCHRA in solution while ZEN adsorption was pH independent. Maximum adsorption was obtained with the highest level of CP present at the zeolitic surface for both mycotoxins.

INTRODUCTION
Mycotoxins are secondary metabolites produced by certain fungi that accumulate in maturing corn, cereals, and other food and feed crops. The most prevalent mycotoxins are aflatoxins, ochratoxins, fumonisins, zearalenone and the trichothecenes. Ochratoxin A (OCHRA) is a mycotoxin produced by *Aspergillus* and *Penicillium* species. It can cause nephrotoxicity, hepatotoxicity, neurotoxicity, teratogenicity and immunotoxicity in both human and animals. Chemical structure of OCHRA is presented in Figure 1(a). Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by *Fusarium* species. Chemical structure of ZEN is presented in Figure 1(b). Due to its structural similarity to the naturally-occurring estrogens, it has strong estrogenic activity and causes infertility and abortion in livestock [1].



(a)



(b)

Figure 1. Chemical structures of OCHRA (a) and ZEN (b).

The most feasible and economical detoxification method is the use of adsorbents as feed additives. The aim is to sequester mycotoxins in the gastrointestinal tract avoiding their absorption by the organism. The natural aluminosilicate minerals, such as montmorillonite or zeolite, effectively bind aflatoxins *in vitro* and *in vivo* conditions. Nevertheless, the binding efficiency of these hydrophilic, negatively charged aluminosilicates to relatively non-polar and hydrophobic mycotoxins such as OCHRA and ZEN is low [2]. Modification of minerals with long chain organic cations (surfactants) results in increased hydrophobicity of their surfaces and consequently higher affinity for hydrophobic organic molecules. Cetylpyridinium chloride (CP) is a surfactant widely used in pharmaceutical and cosmetic formulations as an antimicrobial preservative. It possesses a pyridine head group and a C16 alkyl chain.

In previous studies it was reported that modification of the natural zeolite clinoptilolite with different levels of surfactants – octadecyldimethylbenzyl ammonium chloride (ODMBA) or benzalkonium chloride (BC) increased adsorption of OCHRA and ZEN compared to unmodified clinoptilolite [3,4]. It was determined that adsorption of both mycotoxins increased with increasing amounts of surfactant at the zeolitic surface and that surfactants are active sites at which both mycotoxins are adsorbed.

Since adsorption of mycotoxins may be dependent on the type of surfactant, the aim of this research was to investigate if the natural zeolite modified with CP ions would be also efficient to adsorb OCHRA and ZEN.

METHODS

Starting material was a zeolite-rich tuff from the Zlatokop deposit (Vranje, Serbia) containing primarily clinoptilolite (69%), with the smaller amounts of plagioclase and quartz. The cation exchange capacity (CEC) of the zeolitic tuff was 146 meq/100g, while the external cation exchange capacity (ECEC) was 10 meq/100g. The organozeolites were prepared by the following procedure: 10 g of zeolite was mixed with 100 mL of distilled water containing CP amounts equivalent to 20, 50 and 100% of its ECEC. The suspensions were stirred 10 min at 5000 rpm, and afterwards filtered, washed and dried at 60°C. Samples were denoted as ZCP-20, ZCP-50 and ZCP-100.

OCHRA and ZEN were obtained from Sigma-Aldrich Co. *In vitro* adsorption experiments were performed using the following procedure: duplicate aliquots of 0.1 M phosphate buffer (adjusted to pH 3 and 7) containing 2 ppm OCHRA or ZEN in solution (10 mL) were added to 15 mL Falcon polypropylene tubes to which had been added 20, 10, 5 or 2 mg of each adsorbent. The suspensions were shaken for 30 min at room temperature, then centrifuged at 13000 rpm for 5 min and 2 mL of the aqueous supernatant was removed for HPLC analysis. The percentage of OCHRA and ZEN bound was calculated from the difference between the initial and final concentration in the aqueous supernatant after equilibrium.

RESULTS AND DISCUSSIONS

The organozeolites were obtained by cation exchange of inorganic cations at the zeolitic surface with CP surfactant.

OCHRA is a hydrophobic molecule which possesses carboxylic and phenolic functional groups (Figure 1a). Based on the dissociation constants of OCHRA, $pKa_1 = 3.5$ (carboxylic group) and $pKa_2 = 7$ (phenolic group), OCHRA is present in solution mainly in the anionic form at pH 3 and completely in the anionic form at pH 7. From chemical structure of ZEN (Figure 1b), it is evident that ZEN is a diphenolic compound with an estimated $pKa_1 = 7.62$, suggesting that at pH 3 ZEN is mainly in the neutral form, while at pH 7 the phenolate anion is present in solution [4].

Previous results of OCHRA and ZEN adsorption by unmodified zeolite ($C_0 = 2$ ppm, $C_{susp} = 4$ g/L) showed 40% OCHRA adsorption and 7% ZEN adsorption at pH 3, and 3% OCHRA adsorption and 7% ZEN adsorption at pH 7 [3].

Results for OCHRA and ZEN adsorption by ZCPs at the constant initial mycotoxin concentration (2 ppm) and different amounts of solid phase in suspension at pH 3 and 7 are presented in Figures 2 and 3.

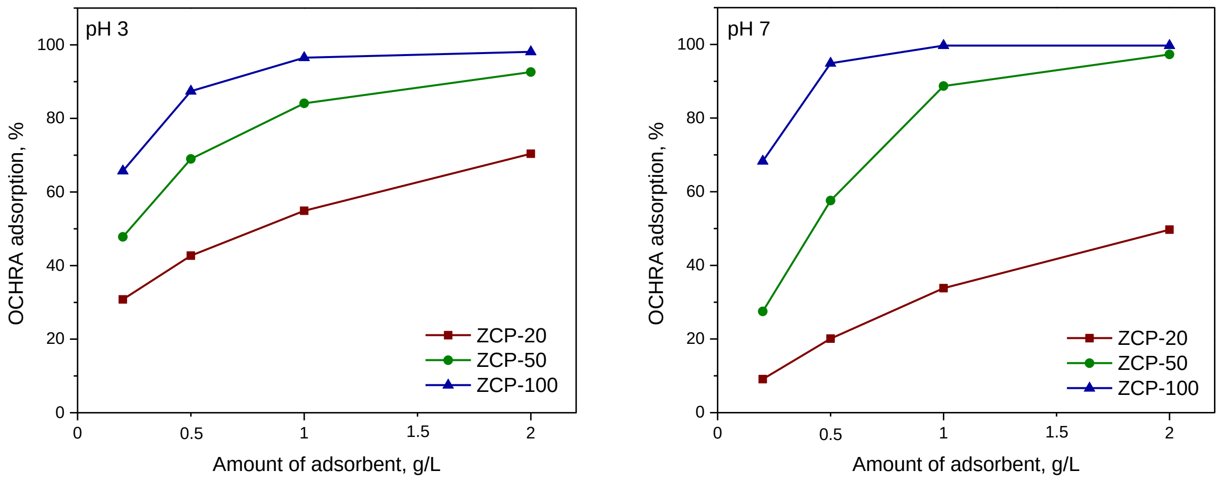


Figure 2. OCHRA adsorption by organozeolites (ZCP-20, ZCP-50 and ZCP-100) at pH 3 and 7.

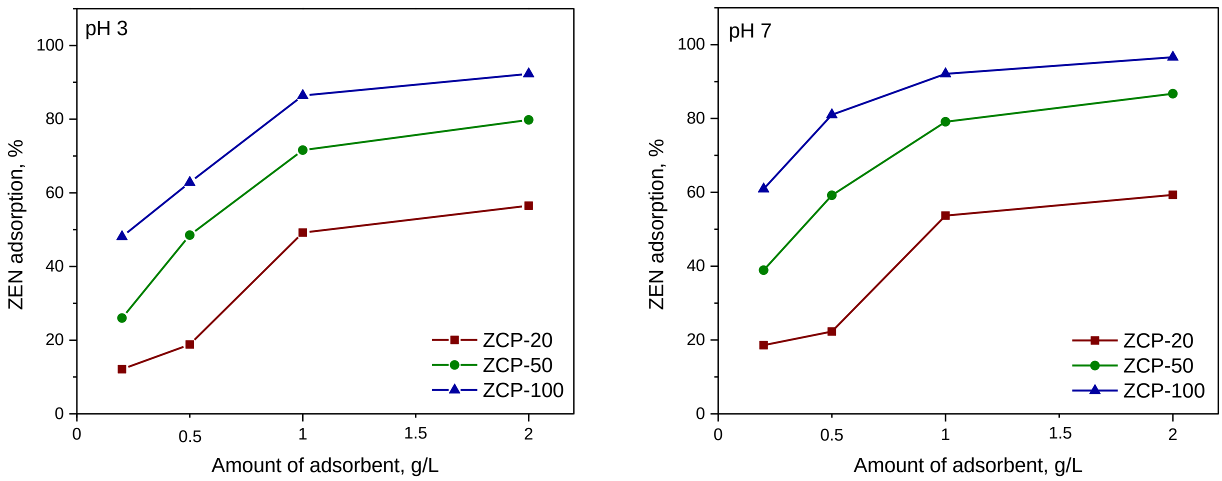


Figure 3. ZEN adsorption by organozeolites (ZCP-20, ZCP-50 and ZCP-100) at pH 3 and 7.

Presence of CP at the zeolitic surface significantly increased adsorption of both mycotoxins. At both pH values, the percentage of OCHRA and ZEN adsorption by ZCPs increased with increasing the amount of each adsorbent in suspension. This increase in adsorption of both mycotoxins can be interpreted with increasing number and availability of adsorption sites at the zeolitic surface. Also, at the same amount of solid phase in suspension, adsorption of OCHRA and ZEN increased with increasing the amount of surfactant at the zeolitic surface. For the lowest level of surfactant, ZCP-20, adsorption of OCHRA was much higher at pH 3 compared to pH 7. With increasing levels of surfactant at the zeolitic surface, differences in OCHRA adsorption at different pH values were less obvious. Compared to the previous results on OCHRA adsorption by unmodified zeolite it is obvious that some active sites are present at the zeolitic surface at pH 3 and together with CP surfactant are responsible for its adsorption. At pH 7, OCHRA is completely in the anionic form and due to the repulsion of the anionic OCHRA and negatively charged zeolitic surface only interactions to CP take place. ZEN adsorption by ZPCs showed similar values at pH 3 and 7 indicating that adsorption was independent of the form of ZEN in solution and that only CP ions present at the zeolitic surface are responsible for ZEN adsorption.

It was previously mentioned that adsorption of mycotoxins by organozeolites may be dependent on the type of organic cation used for the modification of the zeolitic surface. Adsorption of ZEN and OCHRA was previously studied with zeolites modified with the same amounts of ODMBA [3] and BC [4] ions. Since the differences in adsorption of both mycotoxins are more visible when the amount of surfactant at the zeolitic surface is low, results on adsorption of ZEN and OCHRA by organozeolites with the lowest amount of all three surfactants (20% of ECEC value of zeolite) are compared. Organozeolite modified with ODMBA was denoted as OZ-2, while with BC was denoted as BZ-2 [3, 4].

For OCHRA, with the amount of adsorbent in suspension of 2 g/L, the following adsorption indexes were obtained: 70% for OZ-2, 60.9% for BZ-2 and 70.4% for ZCP-20 at pH 3, and 50% for OZ-2, 37.6% for BZ-2 and 49.7% for ZCP-20 at pH 7. This suggests that ZCP-20 was equally efficient as OZ-2, while BZ-2 was slightly less efficient to adsorb OCHRA.

At the same amount of adsorbent in suspension, ZEN adsorption was 72% for OZ-2, 61.9% for BZ-2 and 56.5% for ZCP-20 at pH 3. At pH 7, ZEN adsorption was 79%, 66.4% and 59.3% for OZ-2, BZ-2 and ZCP-20, respectively. This indicates that OZ-2 was more efficient for ZEN adsorption than BZ-2 and ZCP-20. Results confirmed that adsorption of both ZEN and OCHRA is dependent on the type of surfactant used for modification.

CONCLUSION

The organozeolites were prepared by treatment of the natural zeolite clinoptilolite with different levels of CP and *in vitro* adsorption of OCHRA and ZEN at pH 3 and 7 was studied. Increased adsorption of OCHRA and ZEN with increasing amounts of surfactant confirmed that CP at the zeolitic surface is responsible for mycotoxin adsorption. Differences in OCHRA adsorption at different pH values showed that adsorption is dependent of the form of OCHRA in solution and that some active sites are present at the uncovered zeolitic surface. Adsorption of ZEN was pH independent suggesting that only interactions between CP and ZEN are responsible for its adsorption. Results showed that zeolite modified with CP could be good adsorbent as zeolites modified with ODMBA and BC for both OCHRA and ZEN.

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