

# APPLICATION OF MODIFIED NATURAL ZEOLITE PHILLIPSITE FOR REMOVAL OF OCHRATOXIN A

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## ABSTRACT

Organozeolites were prepared by modification of the natural zeolite – phillipsite with surfactant - cetylpyridinium chloride (CP) in amounts of 50, 100, 150 and 200% of the external cation exchange capacity (ECEC) of zeolite. Adsorption of mycotoxin - ochratoxin A (OCHRA) was followed at different solid to liquid ratio, different initial mycotoxin concentration and different pH values. Results showed that adsorption of OCHRA increased with increasing of the initial mycotoxin concentration, the amount of each adsorbent in suspension and with increasing amounts of CP in the organozeolites. For all adsorbents, the adsorption of OCHRA was higher at pH 3 than at pH 7. The highest adsorption was achieved with the organozeolite with the highest content of CP (200% of ECEC), 18.4 mg/g at pH 3 and 8.5 mg/g at pH 7.

**Key words:** zeolite, phillipsite, surfactants, ochratoxin A, adsorption

## INTRODUCTION

Natural zeolites have an affinity to adsorb inorganic cations, but due to the hydrophilic surface and net negative charge, they exhibit low adsorption of fairly nonpolar molecules or anions. In order to increase adsorption of these species, the surface of zeolites is usually modified with long chain organic cations - surfactants. Depending on the amount of surfactant used for modification, the zeolitic surface charge can be changed from negative to neutral (when the amount of surfactant is below or equal to the external cation exchange capacity (ECEC) of the specific zeolite) providing sites for adsorption of fairly nonpolar molecules. However, when the amount of surfactant is above the ECEC value, hydrophobic interactions between alkyl chains of surfactant take place forming a bilayer. In this case, the zeolitic surface is positively charged with a high affinity for adsorption of fairly nonpolar molecules but also anions [1].

Mycotoxins are secondary metabolites of several fungi and are important environmental and carcinogenic agents occurring in many parts of the world [2]. Several approaches have been applied to reduce the contamination of animal feed contaminated with mycotoxins. The most widely used strategy is the addition of so-called mycotoxin adsorbents (commonly zeolites or clays) to animal feed, in order to prevent mycotoxin absorption in the gastrointestinal tract of animals [3]. One of the most common mycotoxin found in contaminated food and feed in Europe is ochratoxin A (OCHRA). It is produced by several fungi of *Penicillium* and *Aspergillus* genera and is recognized as a potent nephrotoxin associated with fatal human kidney disease. According to the International Agency for Research on Cancer (IARC), OCHRA is classified as a possible human carcinogen (2B agent) [4].

In a previous study [5], adsorption of OCHRA was followed on the natural zeolite – phillipsite modified with two surfactants – cetylpyridinium chloride (CP) and

hexadecyltrimethylammonium bromide (HB) in amount equal to the zeolite ECEC value – 100% (PCP-100 and PHB-100). It was determined that both organozeolites significantly increased adsorption of this mycotoxin at pH 3 and 7. Similar OCHRA adsorption capacities were observed for both adsorbents suggesting that its adsorption was not dependent on the type of surfactant used for preparation of the organozeolites. OCHRA is a hydrophobic molecule but also based on the dissociation constants of carboxylic and phenolic functional groups ( $pK_{a1}=3.5$  and  $pK_{a2}=7$ ), it may be present in anionic form at pHs at which adsorption experiments are usually performed. The aim of this research was to further investigate the influence of different amounts of CP present at the phillipsite surface (below and above the ECEC value of the zeolite) on adsorption of OCHRA, at pH 3 and 7.

## EXPERIMENTAL

The natural phillipsite from Neapolitan Yellow Tuff deposit (Campania, Italy), was used for the preparation of organozeolites. The cation exchange capacity (CEC) of the phillipsite was 247.0 meq/100g ( $Na^+=72.0$  meq/100g,  $K^+=103.0$  meq/100g,  $Ca^{2+}=71.0$  meq/100g and  $Mg^{2+}=1.0$  meq/100g), while the ECEC was 13.0 meq/100g [6].

The long chain organic cation - surfactant CP, was purchased from Sigma-Aldrich Co. The organozeolites were prepared by treatment of phillipsite with CP in amounts of 50, 100, 150 and 200% of its ECEC. The detailed procedure for preparation of organozeolites is given elsewhere [7]. Products were denoted as PCP-50, PCP-100, PCP-150 and PCP-200.

Ochratoxin A was supplied from Sigma-Aldrich Co. The effects of amounts of surfactant used to prepare PCPs and adsorbents masses (2.0 and 0.2 g/L) on adsorption of OCHRA were studied at mycotoxin initial concentration of 2 mg/L in 10 mL of buffered solution at pH 3 and 7. Isotherm studies were performed with 5 mg of each adsorbent and 10 mL of buffer solution (pH 3 and 7) containing OCHRA in concentrations from 1.0 to 4.5 mg/L. The pHs were adjusted with small amounts of  $H_3PO_4$  or NaOH. In all experiments, tubes were placed on a rotary shaker for 30 min at room temperature. Then, mixtures were centrifuged and analyzed for the residual mycotoxin concentrations. The initial and residual concentrations of OCHRA were determined by HPLC. Langmuir and Freundlich models were used to fit the experimental data for mycotoxin adsorption by PCPs.

## RESULTS AND DISCUSSION

Organozeolites were obtained by treatment of the natural phillipsite with different amounts of CP. It is well known that when the amount of surfactant is below or equal to the zeolite's ECEC, ion exchange of inorganic cations with surfactant ions occur, while at higher amounts of surfactant a bilayer is formed. Previous results of  $\zeta$ -potential measurements, which is reflection of surface potential, showed that negative surface charge of phillipsite ( $-36.2$  mV) is changed after modification with CP to less negative ( $-36.0$  mV) for PCP-50 and ( $-30.5$  mV) for PCP-100, approached zero ( $-0.8$  mV) for PCP-150 and became positive ( $+10.7$  mV) for PCP-200. Usually, for zeolites, when the amount of surfactant was up to ECEC value,  $\zeta$ -potential became less negative and approached zero indicating the complete monolayer and maximum hydrophobicity of the surface. Bilayer formation at zeolitic surface induces charge reversal causing a positive value of  $\zeta$ -potential. Highly negative value of  $\zeta$ -potential even for sample PCP-100 may be confirmation that CP ions form a local bilayer or micelle at the phillipsite surface rather than a monolayer revealing part of the surface uncovered with surfactants. The value of  $\zeta$ -potential for PCP-150 indicates that for this sample, besides monolayer coverage, patchy bilayer may exist at some parts of the phillipsite surface. Positive value of  $\zeta$ -potential obtained for PCP-200 suggested that additional CP molecules were bonded to the surface via hydrophobic interactions forming a bilayer [7].

Ochratoxin A adsorption by organozeolites at different amounts of each adsorbent and at pH 3 and 7 is presented in Table 1. From Table 1, it can be seen that modification of

phillipsite surface with CP ions significantly increased OCHRA adsorption, since the natural phillipsite exhibited low adsorption of OCHRA (10.0% at pH3 and 3.3% at pH 7 at  $C_{\text{OCHRA}} = 2 \text{ mg/L}$  and  $C_{\text{susp}} = 2 \text{ g/L}$ ). Also, OCHRA adsorption increased with increasing amounts of CP at the phillipsite surface, with the highest adsorption achieved for PCP-200 at both pH values.

Table 1: OCHRA adsorption by organozeolites, at different solid to liquid ratio, at pH 3 and 7.

	OCHRA adsorption, %	
	pH 3	pH 7
PCP-50 (g/L)		
2.0	85.4	82.2
0.2	20.6	15.0
PCP-100 (g/L)		
2.0	94.5	96.7
0.2	37.5	29.1
PCP-150 (g/L)		
2.0	95.5	99.9
0.2	63.1	52.4
PCP-200 (g/L)		
2.0	95.4	99.8
0.2	73.4	65.5

Adsorption of OCHRA by organozeolites was further studied through the determination of adsorption isotherms. Results are presented at Figure 1.

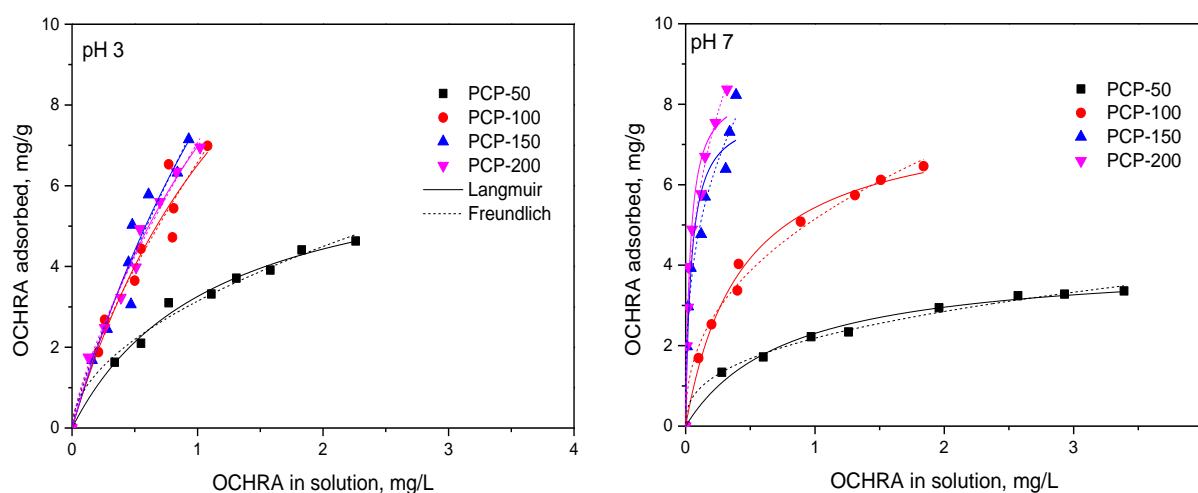


Figure 1. OCHRA adsorption by PCP-50, PCP-100, PCP-150 and PCP-200 at pH 3 and 7

As can be seen from Figure 1, for all organozeolites, OCHRA adsorption increased with increasing of the initial OCHRA concentration following a non linear type of isotherm at pH 3 and 7. High correlation coefficients ( $R^2 > 0.93$ ) were obtained for both Langmuir and Freundlich isotherm models, indicating that these models can be used to simulate OCHRA adsorption on PCPs at pH 3 and 7. It was also observed that adsorption of this mycotoxin

increased with increasing amounts of CP at the phillipsite surface confirming that surfactant ions enhanced its adsorption. Based on the maximum adsorbed amounts of OCHRA from the Langmuir model -  $q_{\max}$ , for all PCPs higher adsorption of OCHRA was noticed at pH 3 than at pH 7, with the highest adsorption achieved for organozeolite containing the highest amount of CP - PCP-200 (18.4 mg/g at pH 3 and 8.5 mg/g at pH 7). Since OCHRA is a hydrophobic molecule that exist partially in anionic form at pH 3 and completely in anionic form at pH 7, thus for organozeolites PCP-50 and PCP-100, probably hydrophobic interactions between hydrophobic part of OCHRA with hydrophobic alkyl tails of CP dominate. At surfaces of PCP-150 and PCP-200 bilayer of CP ions is formed, thus anionic exchange between anionic OCHRA and counter ions from CP bilayer may play an important role in toxin adsorption.

## CONCLUSION

Natural zeolite - phillipsite was modified with four different levels of CP (below and above ECEC value of zeolite) and these organozeolites were used for *in vitro* adsorption of OCHRA. The effects of surface coverage of phillipsite with CP ions, initial OCHRA concentration, solid to liquid ratio and pH of solution on mycotoxin adsorption by organozeolites were investigated. Results showed that the presence of CP ions at the surface of phillipsite increased adsorption of OCHRA at pH 3 and 7. The adsorption of OCHRA by all organozeolites followed a non linear type of isotherm, suggesting a complex adsorption mechanism involving hydrophobic bonding together with electrostatic interactions.

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