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## ADSORPTION OF ZEARALENONE ON SURFACE MODIFIED ZEOLITES - CLINOPTILOLITE AND PHILLIPSITE

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### ABSTRACT

Cetylpyridinium chloride (CP) was used to modify the surface of natural zeolites – clinoptilolite (Z) and phillipsite (P) and adsorption of zearalenone (ZEN) was investigated. Zeolites were modified with two different levels (5 and 10 mmol/100g) of CP and adsorption experiments were done at pH 3 and pH 7. Results showed that with increasing amounts of surfactant at the zeolitic surface, ZEN adsorption increased, as well as with increasing amounts of solid phase in the suspension. At the highest level of surfactant (10 mmol/100g), a slightly higher adsorption of ZEN was observed for phillipsite modified with CP ions, in contrast to clinoptilolite.

Keywords: mycotoxins, zearalenone, adsorption, zeolite, surfactants.

### INTRODUCTION

Zeolites are tectosilicates characterized by an open three-dimensional framework of (Si,Al)O<sub>4</sub> tetrahedra. The tetrahedra form a network of open channels containing molecular water and charge-balancing cations of alkali and alkaline earth metals. Their distinctive crystal structures result in the ability to hydrate/dehydrate reversibly and to exchange cations with aqueous solutions. These properties have widespread industrial applications in water softening, catalysis, remediation of soils and soil quality and in wastewater treatment [1]. In addition, modified natural zeolites can be used to detoxify contaminated animal feed. Animal toxicosis can be caused by mycotoxins (zearalenone, aflatoxins, ochratoxins, fumonisins, trichothecenes, ergot alkaloids) that occur as frequent contaminants of cereal crops [2]. The contamination of foodstuffs and animal feeds is a potential hazard to animal and human health.

Zearalenone (ZEN) is a non-steroidal estrogenic mycotoxin produced by fungi *Fusarium sp.* and it can produce a variety of toxic effects via direct consumption of contaminated cereals and cereal products or indirectly via consumption of animal products. It is carcinogenic, genotoxic and immuno-toxic in both livestock and humans and its toxic effects include infertility, abortion or other breeding problems in livestock [3].

The addition of nutritionally inert mineral sorbents can be successfully used to detoxify animal feedstuff that is contaminated with mycotoxins [4]. Thereby, aluminosilicates, like zeolites and clay minerals lower the bioavailability in the gastrointestinal tract of animals and therefore the toxic effects of mycotoxins. Zeolites are effective in binding aflatoxins under *in vitro* conditions, but less effective in binding other mycotoxins, which are more hydrophobic, such as ZEN. Nevertheless, chemical modification of zeolites with long chain organic cations like octadecyldimethylbenzyl ammonium chloride (ODMBA) or cetylpyridinium chloride (CP) results in an increased hydrophobicity of the zeolite surface, which provides a high affinity for *in vitro* adsorption of hydrophobic ZEN [2, 5].

Cetylpyridinium chloride is a cationic surfactant widely used in industrial and pharmaceutical substances, especially in manufacturing of dermal ointments, drugs and cosmetics [6]. In our previous study it was shown that modification of natural zeolite - clinoptilolite with different levels of CP (2, 5 and 10 mmol/100g) resulted in an increased ZEN adsorption compared to non-modified clinoptilolite [7]. It was demonstrated that with increasing amounts of CP at the zeolitic surface adsorption of ZEN increased. Besides clinoptilolite, other natural zeolites, which may be of interest in biomedical and veterinary applications, are chabazite, mordenite and phillipsite. In this study, the natural zeolite - phillipsite was modified with two different levels of CP (5 and 10 mmol / 100g) and ZEN adsorption was studied under *in vitro* conditions at pH 3 and 7. The results were compared with the results of ZEN adsorption on organoclinoptilolites modified with same amounts of CP. The aim of this study was to investigate if the type of zeolite modified with the same organic cation influence ZEN adsorption.

## EXPERIMENTAL

Two different kinds of zeolite-rich rocks were selected for this study: a zeolite-rich tuff from the Zlatokop deposit (Vranje, Serbia) containing primarily clinoptilolite (Z) [8] and a Neapolitan Yellow Tuff (Campania, Italy) composed primarily of phillipsite (P) [9]. The cation exchange capacity (CEC) quantified by the ammonium chloride method was 146 mmolM<sup>+</sup>/100g, and 247 mmolM<sup>+</sup>/100g for zeolites from Zlatokop deposit and the Neapolitan Yellow Tuff, respectively. Zeolite from the Zlatokop deposit yielded an external cation exchange capacity (ECEC) of 10 mmolM<sup>+</sup>/100g whereas zeolite from Neapolitan Yellow Tuff showed a slightly higher value of 13 mmolM<sup>+</sup>/100g.

The organozeolites are prepared by using 10 g of fine-grained zeolite rich rocks (particle size below 43 μm), and 100 mL of aqueous solution containing cetylpyridinium chloride in amounts equivalent to 50 and 100% of their ECEC. The suspensions are stirred at 5000 rpm for 10 min at 50°C, and afterwards filtered and dried at 60°C. The products of the clinoptilolite-rich rock are denoted as ZCP-5 and ZCP-10 while the products of the phillipsite-rich rock are denoted as PCP-5 and PCP-10.

Zearalenone was obtained from Sigma-Aldrich Co. A primary ZEN stock solution (1000 ppm) was prepared in acetonitrile. ZEN solutions for *in vitro* adsorption experiments were prepared by addition of acetonitrile stock solution to 0.1M phosphate buffer adjusted to pH 3 and 7. The concentration of ZEN used in this study was 2 ppm due to its limited water solubility. Duplicate aliquots of 0.1 M phosphate buffer (pH 3 and 7) containing 2 ppm ZEN in solution (10 mL) were added to 15 mL polypropylene Falcon tubes to which 5 mg and 10 mg of each organozeolite was added. In order to eliminate exogenous peaks, controls were prepared by adding 10 mL of 0.1 M phosphate buffer (pH 3 and 7) to Falcon tubes containing 10 mg of each adsorbent. All samples were placed on a rotator shaker for 30 min at room temperature, centrifuged for 5 min at 13000 rpm and 2 mL of the aqueous supernatant was removed for HPLC analysis. An aliquot of the original buffered ZEN test solution was used as the HPLC standard. The percentage of ZEN bound was calculated by the difference of the initial and final concentration in the aqueous supernatant after equilibrium is reached.

## RESULTS AND DISCUSSION

Natural zeolites - clinoptilolite and phillipsite were modified by cation exchange with CP resulting in organozeolites ZCPs and PCPs.

Preliminary ZEN adsorption results ( $C_{ZEN} = 2\text{ppm}$ , amount of adsorbent 10 mg/10 mL) showed that the adsorption index of non-modified natural zeolite was 7% for clinoptilolite and 15% for phillipsite at pH 3 and 7. Results for ZEN adsorption by natural zeolites – clinoptilolite and

phillipsite modified with different levels of CP at pH 3 and 7, and with different amounts of adsorbents added to ZEN solution are presented in Figs. 1 and 2.

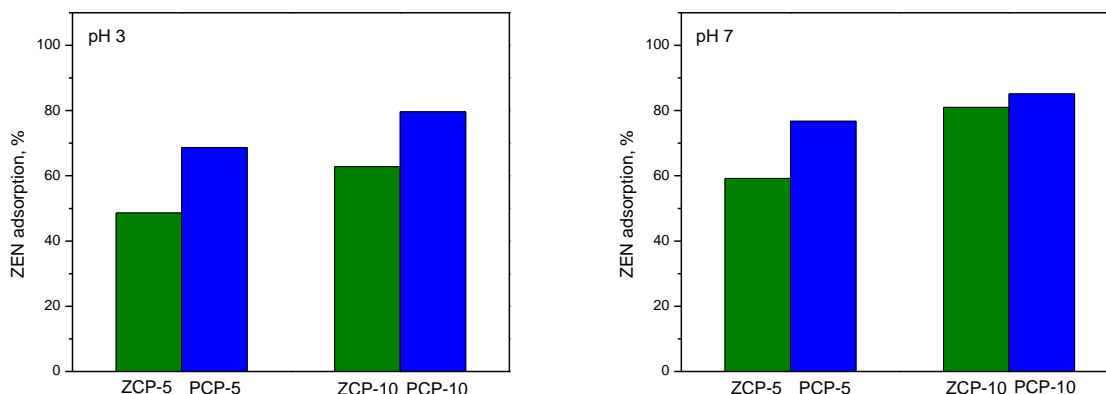


Figure 1. ZEN adsorption by organozeolites ( ZCP-5, ZCP-10, PCP-5 and PCP-10) at pH 3 and 7, when the amount of adsorbent is 5 mg/10mL.

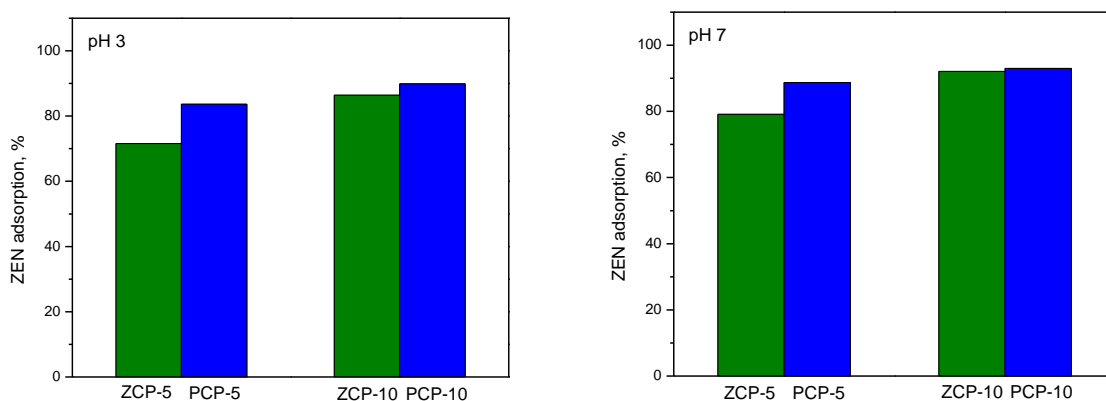


Figure 2. ZEN adsorption by organozeolites (ZCP-5, ZCP-10, PCP-5 and PCP-10) at pH 3 and 7, when the amount of adsorbent is 10 mg/10mL .

As can be seen in Figs. 1 and 2, ZEN adsorption by organozeolites is significantly higher than adsorption by non-modified zeolites. For both organozeolites, ZCPs and PCPs, at pH 3 and 7, adsorption of ZEN increased with increasing amount of organic cation at the zeolitic surface. The higher adsorption of ZEN was achieved when the zeolitic surface was totally covered with CP (ZCP-10 and PCP-10). At pH 3, when the amount of organozeolites added to ZEN solution was 5 mg, ZEN adsorption by ZCPs was: 49% for ZCP-5 and 63% for ZCP-10, while adsorption by PCPs was: 69% for PCP-5 and 80% for PCP-10. At pH 7, the following ZEN adsorption indexes were obtained: 59% for ZCP-5, 77% for PCP-5, 81% for ZCP-10 and 85% for PCP-10. When the amount of organozeolites added to ZEN solution was 10 mg, adsorption indexes at pH 3 were as follows: 72% for ZCP-5, 84% for PCP-5, 86% for ZCP-10 and 90% for PCP-10. At pH 7, with the adsorbent amount of 10 mg, ZEN adsorption indexes were: 79% for ZCP-5, 89% for PCP-5, 92% for ZCP-10 and 93% for PCP-10. These results confirmed that adsorption indexes increased with increasing amount of solid phase in the suspension. Comparing results for phillipsite and clinoptilolite modified with the same level of CP, higher adsorption indexes were observed for phillipsite modified with CP. For all organozeolites a slightly higher adsorption of ZEN was at pH 7, where ZEN exists in solution in the anionic form in comparison to pH 3 where ZEN is mainly in the neutral form. The results showed that

for both organozeolites – clinoptilolite and phillipsite, the degree of hydrophobicity of the zeolitic surface plays an important role in ZEN adsorption. Also the adsorption of ZEN depends on the type of zeolite being used for modification and the higher adsorption of ZEN was achieved with organophillipsites. Results suggested that for both organozeolites – clinoptilolite and phillipsite, long chain organic cations (surfactants) are the active sites responsible for ZEN adsorption.

## CONCLUSION

The organozeolites were prepared by treatment of natural zeolites - clinoptilolite and phillipsite with different levels of cetylpyridinium chloride (CP), surfactant commonly used in the pharmaceutical industry. *In vitro* adsorption of ZEN by these organozeolites was studied at pH 3 and 7. For both types of organozeolites, increased adsorption of ZEN with increasing amount of organic cation at the zeolitic surface confirmed that CP at the both zeolitic surfaces is responsible for ZEN adsorption. Higher adsorption of ZEN was achieved with the organophillipsites at pH 3 and 7. However, to better understand the ZEN adsorption mechanism, more detailed experiments on ZEN adsorption by organozeolites – clinoptilolite and phillipsite will be the subject of future research.

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