



# MME SEE

## CONGRESS 2023

5<sup>th</sup> Metallurgical & Materials Engineering  
Congress of South-East Europe  
Trebinje, Bosnia and Herzegovina  
7-10<sup>th</sup> June 2023

# CONGRESS PROCEEDINGS

**MME SEE**

**C O N G R E S S 2 0 2 3**

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**CONGRESS  
PROCEEDINGS**

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The Faculty of Technology at the University of Banja Luka, Bosnia and Herzegovina;

The Faculty of Metallurgy at the University of Zagreb in Sisak, Croatia;

The Faculty of Natural Sciences and Engineering at the University of Ljubljana, Slovenia;

The Faculty of metallurgy and technology at the University of Podgorica, Montenegro.

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## **PREFACE**

On behalf of the Scientific and Organizing Committee, it is a great honor and pleasure to wish all the participants a warm welcome to the Fifth Metallurgical & Materials Engineering Congress of South-East Europe (MME SEE 2023) which is being held in Trebinje, Bosnia and Hercegovina, 07 - 10 June 2023.

The MME SEE 2023 is a biannual meeting of scientists, professionals, and specialists working in the fields of metallurgical and materials engineering. The aim of the Congress is to present current research results related to processing/structure/property relationships, advances in processing, characterization, and applications of modern materials. Congress encompasses a wide range of related topics and presents the current views from both academia and industry: Future of metals/materials industry in South-East European countries; Raw materials; New industrial achievements, developments and trends in metals/materials; Ferrous and nonferrous metals production; Metal forming, casting, refractories and powder metallurgy; New and advanced ceramics, polymers, and composites; Characterization and structure of materials; Recycling and waste minimization; Corrosion, coating, and protection of materials; Process control and modeling; Nanotechnology; Sustainable development; Welding; Environmental protection; Education; Accreditation & certification.

The editors hope that Congress will stimulate new ideas and improve knowledge in the field of metallurgical and materials engineering. The Congress has been organized by the Association of Metallurgical Engineers of Serbia, with the co-organization of the Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia, Faculty of Technology and Metallurgy, University of Belgrade, Serbia, Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina; the Faculty of Metallurgy, University of Zagreb, Sisak, Croatia; the Faculty of Natural Sciences and Engineering, University of Ljubljana, Slovenia; and the Faculty of Metallurgy and technology, University of Podgorica, Montenegro.

Financial support from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia to researchers from Serbia for attending the congress is gratefully acknowledged. The support of the sponsors and their willingness to cooperate have been of great importance for the success of MME SEE 2023. The Organizing Committee would like to extend their appreciation and gratitude to all sponsors and friends of the conference for their donations and support.

We would like to thank all the authors who have contributed to this book of abstracts and also the members of the scientific and organizing committees, reviewers, speakers, chairpersons, and all the conference participants for their support of MME SEE 2023. Sincere thanks to all the people who have contributed to the successful organization of MME SEE 2023.

On behalf of the 5<sup>th</sup> MME SEE Scientific and Organizing Committee

Miroslav Sokić, PhD

## BENTONITE CLAYS AS AFLATOXIN B<sub>1</sub> ADSORBENTS

Marija Marković<sup>1</sup>, Aleksandra Daković<sup>1</sup>, Danijela Smiljanić<sup>1</sup>, Milena Obradović<sup>1</sup>, Milica Ožegović<sup>1</sup>

e-mail: m.markovic@itnms.ac.rs

<sup>1</sup>Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia

In this study three bentonite clays (bentonite from deposit Beretnica in Republic of Serbia, commercial bentonite and gray clay from Šipovo deposit in Bosnia and Herzegovina) were investigated as adsorbents of aflatoxin B<sub>1</sub> (AFB<sub>1</sub>). In a single in vitro AFB<sub>1</sub> adsorption experiment, all three bentonites showed high adsorption efficiency for AFB<sub>1</sub> at pH 5. The effect of adsorbent mass on AFB<sub>1</sub> adsorption was also studied, with the same initial AFB<sub>1</sub> concentration, at pH 3, and it was shown that toxin adsorption decreased with decreasing amount of each bentonite in suspension. Thermal analysis was performed in order to determine the structural differences between investigated bentonites. Results showed that gray clay was *trans*-vacant bentonite while other two clays were *cis*-vacant bentonites. Aflatoxin B<sub>1</sub> adsorption experiments, at pH 3, showed that *trans*-vacant gray bentonite had higher affinity for AFB<sub>1</sub> than commercial *cis*-vacant bentonite. The highest adsorption was achieved with *cis*-vacant bentonite from Beretnica deposit.

**Keywords:** bentonite, structural configuration, mycotoxins, aflatoxin B<sub>1</sub>, adsorption

### Introduction

Aflatoxins (AFs), a class of mycotoxins produced by fungal species, mainly *Aspergillus flavus* and *Aspergillus parasiticus*, are present in feed or food. AFs, until a few years ago, have not been recognized as contaminants of big concern for primary production in Europe. However, recently, situation is different due to the climate change and in the years 2003 and 2012 in Italy and South Europe, the significant contamination of corn with AFs was reported (Battilani et al. 2016). There are more than twenty different forms of naturally occurring AFs, among which aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is the most toxic. AFB<sub>1</sub> is acutely and chronically toxic to humans and animals, causing liver damage, liver cirrhosis, induction of tumors, and teratogenic effects. Various techniques such as physical treatment, chemical detoxification and biological degradation have been adopted to remove, destroy or reduce AFB<sub>1</sub>, and subsequently protect animal and human health. At present, adsorption approach is considered to have the most potential in treating mycotoxins contaminated feed. Among various adsorbents, clay minerals such as bentonites have drawn global attention due to their high efficiency to adsorb AFB<sub>1</sub>, nature abundance and low cost. Bentonites are composed primarily of montmorillonite which is dioctahedral 2:1 layer aluminosilicate. A layer is built of an octahedral sheet sandwiched between two tetrahedral silicate sheets (Liu et al. 2021).

The mineralogical, chemical and physico-chemical characteristics of bentonites have influence on adsorption of AFB<sub>1</sub>. According to literature, the following characteristics influence AFB<sub>1</sub> adsorption: structural composition of the octahedral sheet (bentonites with higher amount of structural iron and magnesium in octahedral sheet had higher AFB<sub>1</sub> adsorption capacities), charge density (high-charge density bentonites had low adsorption of AFB<sub>1</sub>), the type of exchangeable cations (saturation of bentonites with divalent cations enhanced adsorption capacity and affinity for AFB<sub>1</sub>) and recently, structural configuration of the octahedral sheet (the *cis*-vacant bentonites showed higher AFB<sub>1</sub> adsorption than the *trans*-vacant bentonites) (Tenorio Arvide et al. 2008; Deng et al. 2012; Barrientos-Velázquez et al. 2016; Vekiru et al. 2015).

Bentonite, in the form of dioctahedral montmorillonite, is authorized in the European Union (European Union Regulation 1060/2013) as a technological additive - substance for the reduction of the contamination of feed by AFs, since 2013. According to this Regulation, bentonite should contain ≥ 70% smectite (dioctahedralmontmorillonite), < 10% opal and feldspar and < 4% quartz and calcite (based on XRD analysis). The adsorption capacity of the specific bentonite for AFB<sub>1</sub> needs to be above 90% (European Commission 2013).



In this study two bentonites (one from deposit Beretnica in Republic of Serbia and the other commercial bentonite) were tested as adsorbents for AFB<sub>1</sub> according to the method described by the European Union Regulation. Results were compared with results of AFB<sub>1</sub> adsorption by previously studied gray clay (Shannon et al. 2017; Daković et al. 2008). In order to determine the structure of the octahedral sheet of bentonites, they were characterized by thermal (DSC/TG) analysis. Impact of adsorbent dose on AFB<sub>1</sub> adsorption by all three clays at pH 3 was also investigated.

### Materials and methods

Bentonites used in this study were: bentonite from deposit Beretnica in Republic of Serbia (BC), commercial bentonite supplied from Fagron Co (CC), and gray clay from Šipovo deposit in Bosnia and Herzegovina (GC). AFB<sub>1</sub> was obtained from Sigma-Aldrich Co. Thermal analysis of bentonites was performed on a NETZSCH STA 449 F5 Jupiter. Samples were heated (25-1000°C) in a synthetic air atmosphere with a heating rate of 10°C/min. Before analysis, samples were dried at 60°C for 2 h and kept in a desiccator at a relative humidity of 75% for 24 h. Adsorption of AFB<sub>1</sub> according to the method described by the European Union Regulation was carried out in 0.1 M phosphate buffer at pH 5 with AFB<sub>1</sub> concentration of 4.0 mg/L and with 0.02% (w/v) – 0.2g/L of each adsorbent. The adsorption of AFB<sub>1</sub> by three clays was further followed with the same initial concentration of AFB<sub>1</sub> and with different amounts (0.2, 0.1, 0.05, and 0.02 g/L) of each adsorbent in suspension, at pH 3. Suspensions were shaken for 30 min at room temperature, and then centrifuged at 13000 rpm for 3min. The initial and final concentrations of AFB<sub>1</sub> were determined by high-performance liquid chromatography (HPLC). The Shimadzu HPLC system included a pump LC-20AD, autosampler SIL-20A HT and fluorescence detector RF-20A ( $\lambda_{ex} = 365$  nm,  $\lambda_{em} = 430$  nm). Chromatographic separations were carried out on Phenomenex column (Hyperclone C<sub>18</sub>100 × 4.6 mm, 3 μm particle size), while the mobile phase was methanol:acetonitrile:water (20:20:60) and was pumped at a flow rate of 1 mL/min.

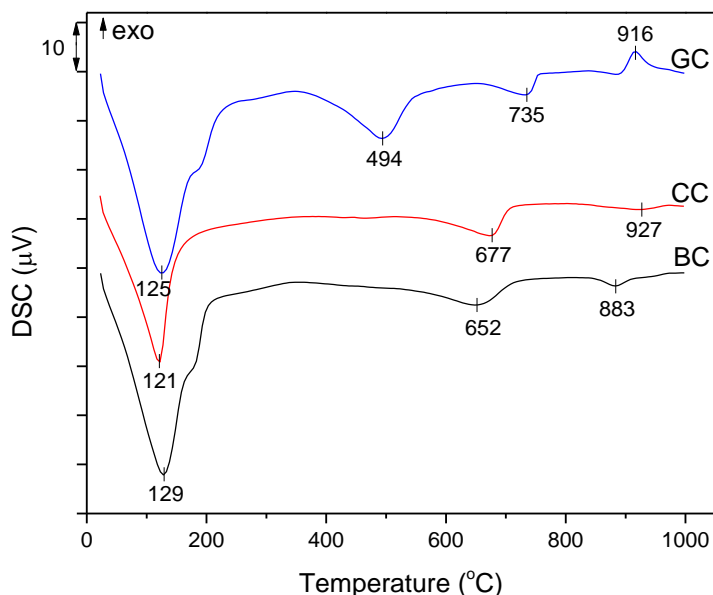
### Results and discussion

The octahedral sheet is the most distinguished structural characteristic of the dioctahedral 2:1 layer aluminosilicate –bentonites and their dehydroxylation behavior is related to the distribution of the metal ions and vacancies in the octahedral sheet. The dehydroxylation peak temperature achieved by thermal analysis can be used to characterize the structure of the octahedral sheet. *Cis*-vacant bentonites dehydroxylate around 650-700°C, while dehydroxylation of *trans*-vacant bentonites occurred at around 500-550°C (Wolters and Emmerich 2007).

Thermal (DSC/TG) analysis was used to study the thermal properties of bentonite clays in order to understand the structure of their octahedral sheets. DSC curves are presented in Figure 1, while the mass losses for all samples in different temperature regions are given in Table 1.

**Table 1** Mass loss from TG curves of bentonite clays

	Massloss (%)			
	25-250°C	250-550°C	550-800°C	$\Sigma$ (25-1000)°C
BC	20.20	1.17	2.25	23.72
CC	14.68	1.09	2.40	18.32
GC	18.49	4.14	2.72	25.41

**Figure 1** DSC curves of bentonite clays

From Figure 1, it can be seen that all three bentonites exhibited endothermic peak around 120°C (125°C for GC, 121°C for CC and 129°C for BC) corresponding to the dehydration of physisorbed water and water coordinated to the metal cations. This process is accompanied by a mass loss of 20.20% for BC, 14.68% for CC and 18.49% for GC, in the temperature range of 25-250°C on TG curves (Table 1). At the DSC curve of GC endothermic peak at 494°C which is associated with the mass loss of 4.14% in the temperature range of 250-550 °C, corresponded to the dehydroxylation of the structural OH groups in bentonite, while dehydroxylation of the CC and BC occurred at higher temperatures, at 677°C for CC and at 652°C for BC with corresponding mass losses of 2.40% and 2.25%, respectively. Based on the dehydroxylation peak temperature, GC is considered as *trans*-vacant bentonite, while BC and CC are *cis*-vacant bentonites. At DSC curve of GC bentonite, endothermic peak at 735°C was visible which originated from the combustion of carbonates. Endothermic peak at 883°C for BC and 927°C for CC, as well as exothermic peak at 916°C for GC, corresponded to the phase transformation of montmorillonite and are not accompanied by any change in mass loss (Bayram et al. 2010; Daković et al. 2008).

Initial results on the adsorption of AFB1 by BC, CC and GC at pH 5 are presented in Table 2. Single AFB1 adsorption experiment at pH 5, according to the European Union approved method, showed that all three bentonites adsorbed more than 90% of AFB1. The highest adsorption of AFB1 was achieved with BC (98.0%), while CC and GC had a slightly less percent of AFB1 adsorbed (91.7% for CC and 92.3% for GC). Further experiments on AFB1 adsorption were performed with the same initial concentration and with different amount of adsorbents (0.2-0.02 g/L), at pH 3. These results are also summarized in Table 2. Results showed that adsorption of AFB1 at pH 3, under the same experimental conditions (AFB1 initial concentration = 4.0 mg/L, adsorbent concentration = 0.2 g/L), was higher in comparison to pH 5 for all three bentonites (99.1% for BC, 93.5% for CC and 95.6% for GC).

It is also visible, that with decreasing of the amount of each adsorbent in suspension, adsorption of AFB1 decreased and differences in AFB1 adsorption efficiency between bentonites became more visible. Thus, at the lowest amount of each adsorbent of 0.02 g/L, AFB1 adsorption was 52.2% for BC, 31.1% for CC and 33.5% for GC.

**Table 2** Adsorption of aflatoxin B<sub>1</sub> by bentonite clays at pH 5 and pH 3

	AFB1 adsorption (%)	
	pH 5	pH 3
BC (g/L)		
0.2	98.0	99.1
0.1		98.1
0.05		88.4
0.02		52.2
CC (g/L)		
0.2	91.7	93.5
0.1		83.8
0.05		62.5
0.02		31.1
GC (g/L)		
0.2	92.3	95.6
0.1		85.1
0.05		67.3
0.02		33.5

Vekiru et al. (Vekiru et al. 2015) tested seven bentonites and one zeolite as adsorbents for AFB1 under *in vitro* and *in vivo* conditions. Six of seven bentonites were *cis*-vacant and one was *trans*-vacant. They reported that under *in vitro* conditions, at pH 5 (European Union approved method), all six bentonites showed high efficiency for AFB1 (adsorption index was equal or above 90.0%), *trans*-vacant bentonite showed moderate adsorption of AFB1 (adsorption index was 63.8%), while by zeolite, adsorption of this toxin was low (8.4%). They concluded that zeolite was ineffective in AFB1 binding, while *cis*-bentonites were more effective than *trans*-bentonite as adsorbents for this toxin. Contrary to their results, adsorption of AFB1 by all three clays, under these conditions, and at pH 3 was over 90%. The differences in AFB1 adsorption were visible at the lowest amount of each adsorbent in suspension. However, although GC is *trans*-vacant bentonite and should have lower adsorption of AFB1 compared to *cis*-vacant bentonite, results showed that among all investigated bentonites, the lowest adsorption of AFB1 was achieved with CC which is a *cis*-vacant bentonite. Results indicated that structural configuration of bentonite is not the main adsorbent property influencing the AFB1 adsorption capacity. Further characterization of materials, as well as detailed AFB1 adsorption experiments (determination of adsorption isotherms) will be performed in order to define mechanism of AFB1 adsorption by these bentonite clays.

## Conclusion

Three bentonite clays were tested as adsorbents for AFB1. All three bentonites possess high adsorption efficiency for AFB1 according to the method described by European Union Regulation. Characterization of clays by using thermal analysis showed structural differences between investigated bentonites. The BC and CC are considered as *cis*-vacant bentonites, while the GC is *trans*-vacant bentonite. Although the *trans*-vacant bentonite was expected to have the lowest AFB1 adsorption, the lowest adsorption of AFB1 was achieved with CC, a *cis*-vacant bentonite. Between investigated bentonites, the highest adsorption of AFB1 was achieved with BC at pH 3.

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