Published with Open Access at Journal BiNET
Vol. 03, Issue 01: 135-147
Journal of Molecular Studies and Medicine Research
Journal Home: www.journalbinet.com/jmsmr-journal.html

An in-vitro evaluation of the capacity of crude clay and ash based materials to bind aflatoxins in solution

Ayo E. M.[1*], A. Matemu[1], G. H. Laswai[2] and M. E. Kimanya[1,3]

[1]The Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
[2]Sokoine University of Agriculture, Morogoro, Tanzania
[3]Dept. of Rural Economy and Agriculture, African Union Commission, Addis Ababa, Ethiopia.

✉ Corresponding author*: ayoe@nm-aist.ac.tz
Article received: 27.06.18; Revised: 14.08.18; First published online: 25 October 2018.

ABSTRACT

Aflatoxins cause health burden in feed chain particularly in tropical areas of the world, causing great health hazards to animals and in advance, to human. Currently, there is no universal measure to detoxify aflatoxins in contaminated feeds to render them safe. The study aimed to evaluate potential of crude clays and ashes as test binding materials (TBM) in binding aflatoxins in solution, towards reducing their toxicity to animals ingesting aflatoxin contaminated feeds. Using in-vitro technique, clays designated AC, KC, CC and MC and ashes VA and RA were evaluated for their capacity to bind aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1) and G_2 (AFG_2) relative to a commercial binder Mycobinder^R (Evonik) as a reference. On average, CC, VA, KC, MC, AC, RA and Mycobind^R were able to bind 39.9%, 51.3%, 61.5%, 62.0%, 72.6%, 84.7%and 98.1% of the total aflatoxins in buffered solution, respectively. The capacity of AC and RA was statistically ($p<0.05$) equal and potentially high in binding aflatoxins next to the Mycobind^R. The capacity trend of the TBM and Mycobind^R to bind aflatoxins, largely seemed to follow the trend of their cation exchange capacity (CEC). The CEC (meq/100g) varied as 7.0, 15.4, 18.8, 25.4, 27.2, 27.2 and 38.9 for CC, MC, KC, VA, AC, RA and Mycobind^R, respectively. The Average proportions of AFB1, AFB_2, AFG_1 and AFG_2 adsorbed to the TBMs were 96.3%, 42.7%, 80.8% and 32.1%, respectively. The binding capacity of the TBM relative to Mycobind^R was about 100% for AC and RA, 50% for KC, MC and VA and 33.3% for CC. The AC and RA seem to be promising resources in binding aflatoxins and can be utilized in alleviating aflatoxin contamination of feeds.

Key Words: *Clays, Ashes Aflatoxins, Binding capacity, In-vitro and Contaminated feeds*

Cite Article: Ayo E. M, Matemu, A., Laswai, G. H. and Kimanya, M. E. (2018). An in-vitro evaluation of the capacity of crude clay and ash based materials to bind aflatoxins in solution. Journal of Molecular Studies and Medicine Research, 03(01), 135-147. Crossref: https://doi.org/10.18801/jmsmr.030118.16

I. Introduction

Aflatoxins are natural toxins produced in foods and feeds, primarily, by certain species of fungi, specifically *Aspergillus flavus* and *Aspergillus parasiticus,* when conditions are favourable for fungal growth and subsequent toxin formation. Aflatoxins exist in four forms of health, agricultural and

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

economic importance, namely aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2) (Lopez *et al.* 2002; Dhanasekaran *et al.* 2011; Jen *et al.* 2017). The most toxic and abundant aflatoxins is AFB1 (Feddern *et al.* 2013). Almost all feed resources contain certain levels of naturally occurring aflatoxins and any level of dietary aflatoxins poses a certain level of health risk (Sassahara *et al.* 2005). Studies show that aflatoxins in feeds depress growth and production performance of animals (Andretta *et al.* 2012; Mok *et al.* 2013). When animals are fed naturally aflatoxin-contaminated feeds, the toxins (mostly AFB1) are secreted in cow milk or retained in hen eggs as aflatoxin M1 (AFM1) (Khan *et al.* 2013; Grace, 2013; Arapcheska *et al.* 2015). It is therefore imperative to prevent and reduce hazards of aflatoxin contamination of feeds for protection and promotion of human and animal health. Some of the techniques used to reduce aflatoxin contamination of feeds are thermal inactivation and irradiation as physical techniques and treatment of the feeds with acidic or alkaline solutions, ozone treatment and ammoniation as chemical techniques and detoxification by microbial agents as biological techniques (Diaz and Smith, 2005; Kolosova and Stroka, 2012). These techniques are mostly applied in the animal industry and are reported to have some limitations including costs implications, requirement of some complicated facilities, reduction of dietary palatability and nutritional values, also creating danger of unsafe chemical residual (Devreese, 2013). Techniques involving toxin binders (also called adsorbents or sequesters) have been employed owing to their economic feasibility, applicability and nutritional safety. Many types of crude or refined materials including clays, cellulose products, yeast cell wall products and activated charcoal products are envisaged to have ability to sequester or bind aflatoxins (Phillips *et al.* 1995; Phillips *et al.* 2002; Vekiru *et al.* 2015). The potential binding capacity of these materials are known to vary depending on their nature and source (Vekiru *et al.* 2015). According to Kannewischer *et al.* 2006; Vekiru *et al.* 2007 (cited by Vekiru *et al.* 2015) there is no existing clear generic linear relationship between binding effectiveness and specific adsorbent properties, such as elemental and mineralogical content, cation exchange capacity (CEC) and pH levels of materials. The binding potential of some materials particularly clays seems to be the function of their chemical composition, such as Ca^{+} and K^{+} ions present in the framework configured by Silicon, Aluminium and Iron oxide. Studies show that Alumino-silicates have wide variation of these elements (Table 01). In South American countries, ashes such as soda ash and wood ash have been used in some food processes such as in niztamalization for corn tenderization where dietary aflatoxins load is also reduced owing to breakage of aflatoxin lactone-ring by the ash alkalinity (Moreno-Pedraza *et al.* 2015). In Tanzania, farmers are using an imported binder that has proven to be useful in terms of protecting livestock from aflatoxin exposure. However, the imported binders are expensive; the high cost of importing these products which are clay-based materials can be avoided if local resources and sources with similar potential are identified. Our experience in animal husbandry in Tanzania shows that there is a number of clay and ash based materials directly eaten by human or added to feeds and foods for various purposes.

Table 01. Percent structural components in clay samples and Rice-husk ashes collected from various locations

#of samples	Mean/Range	Percent structural components of claysand RHA samples					**Source**
		SiO_2	Al2O3	Fe_2O_3	CaO	K_2O	
11 clays	Mean	59.6	19.0	5.2	1.7	0.8	Karnland (2010)
	Range	1.1-69.0	0.5-21.7	0.2-14.8	0.1-6.8	0.1-3.3	
10 clays	Mean	55.3	13.7	4.4	1.4	1.3	Mukasa-Tebandeke *et al.* (2015)
	Range	44.3-71.0	8.4-20.1	1.4-8.0	0.1-2.4	0.1-2.6	
		80.2	13.2	2.7	0.2	0.1	Anjos *et al.* (2016)
RHA	Mean	88.3	0.5	0.7	0.7	2.9	Habeeb and Mahmud (2010)
RHA	Mean	89.0	1.2	1.3	1.0	1.2	Mohamed *et al.* (2015)
RHA	Mean	93.4	0.1	0.1	0.3	1.4	Korotkova *et al.* (2016)

RHA: Rice-husk ash

The clay-based materials are sold for geophagia purposes, mostly demanded by some groups of women especially pregnant ones. Ashes have been used in traditional cookery of some local foods such as corn recipes and in feeds as ration improvers or appetisers. We hypothesize that these materials could provide aflatoxin binding capacity equivalent to the imported product. Arbitrarily, we selected clays obtained in the regions of Arusha, Kilimanjaro, Morogoro and Coast and also volcanic ash and rice husk ash from Arusha region. The potential of these materials in binding toxins has been speculated from the instinct of among animals, birds and human eating soils, which shield them from toxic effects of some ingested natural toxins (Diamond, 1999; Mahaney and Krishnamani, 2003). The objective of the study was therefore to evaluate the chemical composition and the capacities of the above-mentioned materials in binding aflatoxins.

II. Materials and Methods

Test binding materials and their sources: Six crude test-binding materials (TBM) were evaluated against a reference binder Mycobind^{R}. The binding materials were four clays, designated AC, KC, CC and MC and two ash-based materials volcanic ash (VA) and rice-husk ash (RA). Nature, source and ethno-utilization of the TBM are shown in Table 02. Samples of AC, KC, CC, and MC were taken by taking about ten bits of each from various parts of lots at the source site to make a representative sample of about 5kg.The sample of each TBM was taken to laboratory for preliminary grinding, sieving, homogenizing and then packed in zip bags for subsequent analyses and evaluation in the experiment.

Table 02. Physical appearance, sources and current uses of the test materials

Material ID	Physical appearance	Source region	Ethno-utilization
Clays			
AC	Brick-red clogs	Arusha	Treatment of human skin infection and ailments
KC	Brownish-red blocks	Kilimanjaro	Geophagial
CC	Shiny white granules	Coast	Stomach ailment treatment and for decorations.
MC	Brownish-red granules	Morogoro	Geophagial
Ashes			
VA	Greyish Volcanic powder	Arusha	Food seasoning and tenderization in traditional cookery, feed additive
RA	Greyish-white fine powder	Various	Fertilizer

Five kilogram of VA was purchased from the market and the site of production was followed to ascertain its originality, and then handled like for the clay TBM in the laboratory. Representative sample of rice husks was taken from rice-millers and incinerated in the laboratory furnace at a temperature of 550^{o}C for four hours and was used to make about 5kg of rice-husk ash.

The reference binder: For comparison of the binding capacity of the crude clays and ashes, a commercial mycotoxin detoxifier named Mycobind^{R} (Evonik Industries AG) was employed. The MycobindR was purchased from Farmers Centre Limited in Dar es Salaam, Tanzania.

Aflatoxin solution: The stock solution of aflatoxins produced by Romer Labs, Inc. USA was donated by Tanzania Food and Drugs Authority (TFDA).

Chemical analysis of the test materials: Samples of the TBM/Mycobind^{R} were further homogenized, ground and sieved through 1mm sieve for the subsequent analyses of mineralogical composition, elemental content and cation exchange capacity (CEC).

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

Mineralogical composition: Samples of the TBM/Mycobind^{R} were analysed for mineralogical composition using non-destructive techniques that employed X-RD analyser (BTX SN 231, Olympus Corporation, Tokyo Japan), a self-calibrated diffractometer depending on temperature. The samples were analysed at a temperature of -45^{o}C. About 15mg of finely ground sample was sieved through 150µm sieve and loaded in the vibrating sample holder of the X-RD analyser for scanning. The results were XRD-spectrum patterns received on a screen of a computer connected to the analyser showing peaks corresponding to each specific mineral present in the sample.

Elemental-oxide composition: The oxides in the TBM/Mycobind^{R} were quantified by Minipal-4 a high performance bench top energy dispersive X-ray fluorescence spectrometer (PANalytical MINIPAL-4, EDXRF Spectrometer, The Netherlands).The sample was ground into a fine powder, then about 50g of it was scanned by the spectrometer for metallic oxide composition at an energy dispersion of 30keV. The percent composition of the metallic oxides in each sample was recorded.

Determination of cation exchange capacity (CEC): The CEC was determined by wet analysis employing Ammonium Replacement Method (Buchner funnels vacuum flasks) as explained by Brady and Weil (1990) and involving leaching of exchangeable cations in the TBM/Mycobind^{R} with ammonium acetate salt solution. The excess salt was removed by ethanol followed by potassium chloride to leach NH_4^+which initially replaced other various cations of the TBM/Mycobind^{R}. The amount of $NH4^+$ released and washed into a beaker beneath Buchner funnels was determined using Kjeldahl distillation method (Bremner, 1960) and CEC (meg/100g) of TBM/Mycobind^{R} was computed as:

CEC = (mg L^{-1} of NH_4-N in leachate) x (0.25/14) x (100/sample weight (g)) mg L^{-1} NH_4-N.

Experimental design and treatments

Experimental design: The six TBM and Mycobind^{R} engaged to bind aflatoxins formed seven treatments of the in-vitro experiment. Each of the treatments was replicated into three units (test-tubes).

Preparation of the experimental solutions: The experiment was based on a buffer solution with or without a TBM/Mycobind^{R} and spiked or non-spiked with aflatoxin solution.

a. **Buffer solution:** The buffer solution was prepared from Potassium Chloride, Potassium dihydrogen phosphate anhydrous disodium hydrogen phosphate and Sodium chloride in distilled water
b. **Diluted aflatoxin solution:** The standard solution of combined aflatoxins AFB1, AFB2, AFG1and AFG2 (250ng/ml) in acetonitrile was diluted to 20ng/ml using distilled water in an amber flask.

c. **Solutions of TBM/Mycobind^{R} and controls:** The test solutions contained components as shown and summarised in Table 03.

(i) Spiked TBM/Mycobind^{R}: suspension of 0.25% of TBM/Mycobind^{R} in the buffer solution spiked with 5ml of diluted solution of aflatoxins
(ii) Non-spiked TBM/Mycobind^{R}: a control for each binding material containing suspension of 0.25% of TBM/Mycobind^{R} in the buffer solution.
(iii) Positive control: the buffer solution spiked with 5ml of diluted solution of aflatoxins and
(iv) Negative control: the buffer solution only.

For each solution, three replications were taken in separate test tubes as experimental units.

Procedure for *in-vitro* experiment: The *in-vitro* procedure was adopted from Kong *et al.* (2014) simulating gastrointestinal pH condition of pigs, representing monogastric animals, which are more prone to aflatoxicosis. A sample of TBM/Mycobind^{R} was prepared by weighing 0.025g into 10ml of phosphate buffer solution (0.1 M, pH 6.0) making a suspension of 0.25%. An aliquot of 2.5 suspension was pipetted into 25 ml centrifuge-tube then 5 ml of the diluted aflatoxin solution was added. Parallel with the TBM/Mycobind^{R} test treatments, their respective negative controls (non-spiked with the diluted aflatoxin solution) were ran. General positive and negative controls were included to eliminate probable error effects such as due to aflatoxin impurities in the measuring/analysis system hardware and reagents. The positive control contained 2.5ml of phosphate buffer and 5ml of the diluted aflatoxin solution while the negative control contained5 ml of phosphate buffer solution only. Each solution

sample was replicated thrice and pH in each centrifuge-tube was adjusted to 2.0 by adding 1M HCl to simulate pH in the stomach of pigs.

Table 03. Experimental Solution Samples

Solution samples	Composition	#of samples	Replications	Total # of units (tubes)
Spiked TBM/Mycobind^R	Buffer solution, TBM/Mycobind^R and diluted aflatoxin solution	7	3	21
Controls				
Non-spiked TBM/Mycobind^R	TBM/Mycobind^R and buffer solution	7	3	21
Positive control	Buffer solution and diluted aflatoxin solution	1	3	3
Negative control	Buffer solution	1	3	3

Incubation of the solution samples: All samples were incubated at39°C in a shaking water bath for two hours and then1ml of phosphate buffer (0.2 M, pH 6.8) was added to each tube. To simulate the conditions in the small intestine of pigs, pH in all tubes was raised to 6.8 by adding 1M NaOH followed by second phase incubation at 39°C for four hours. After incubation, the mixture was centrifuged and the supernatant was obtained for analysis of residual (unbound) aflatoxins B1, B2, G1 and G2 using High Performance Liquid Chromatography (HPLC).

Determination of unbound aflatoxins in the buffer solution: Briefly, the pH of the clear supernatant was adjusted to 7.4 using 0.1M NaOH. Unbound aflatoxin in the supernatant was determined by the procedure suggested by Diaz *et al.* (2003), where the clear supernatant was analyzed for residual (unbound) aflatoxin without additional cleanup. The analysis employed fluorescence detector connected to HPLC (Shimadzu Corp) at a mobile phase flow rate of 0.8ml/min at a temperature of 28^{o}C, through stationary phase column of size 5µm 4.6x150mm (Spherisorb ODS-1, Waters).Residual aflatoxins AFB1, AFB2, AFG1and AFG2 were quantified at wavelengths of 363nm excitation filter and 440nm cut-off emission filter using the fluorescence detector (RF-10AXL SMN C20954406285).

Estimation of percent aflatoxin binding capacity: Aflatoxin binding capacity of a material was determined by the percent of AFB1, AFB2, AFG1 or AFG2 adsorbed into it. Thus, the higher the aflatoxin binding capacity the lower the percent of unbound aflatoxin content in the buffer solution. The Percent binding capacity P_i of i^{th} TBM/Mycobind^R in binding j^{th} aflatoxin was determined using the formula (1).

$$P_i=(IAT_{ij}- UAT_{ij})/IAT_{ij}\times100 \ldots\ldots\ldots\ldots (1)$$

Where, IAT_{ij} (ng/ml) is the initial concentration of j^{th} aflatoxin in the test-tube with i^{th} TBM/Mycobind^R; UAT_{ij} (ng/ml) is the residual (unbound) j^{th} aflatoxin in the test-tube with i^{th} TBM/Mycobind^R after the digestion period. The IAT_{ij} was considered to be the amount of aflatoxin recovered from positive control adjusted by subtracting the value obtained for the negative control. The UAT_{ij} was adjusted by subtracting residual aflatoxin amount obtained for the negative control of each individual TBM/Mycobind^R from the concentration of residual aflatoxin in the supernatant of theTBM/Mycobind^R spiked with aflatoxin solution.

Statistical analyses: Data analysis for percent mean binding capacity were analysed by GLM program of SAS (SAS, 2004) using the model formula (2).

$$Y_{ij} = X_i + X_j + e_{ij} \ldots\ldots\ldots\ldots (2)$$

Where, Y_{ij} =binding response (capacity) of i^{th}TBM/Mycobind^R in adsorbing j^{th} aflatoxin
X_i =binding effect due to the capacity of i^{th}TBM/Mycobind^R in adsorbing j^{th} aflatoxin

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

X_j =binding effect due to easy with which j^{th} aflatoxin is adsorbed to i^{th} TBM/Mycobind^R
e_{ij} =the error term due toi^{th} and j^{th} aflatoxin

The mean separation was done by Duncan procedure and the significance was declared at an alpha-level of 0.05.

Relationship between binding capacity of TBM/Mycobind^R in adsorbing aflatoxins and their chemical properties was determined by running correlation analysis between percent binding capacity of TBM/Mycobind^R and their relative chemical properties (elemental-oxide concentration and cation exchange capacity). MS-Excel was employed in this analysis.

Determination of aflatoxin binding capacity ratio of Mycobind^R to TBM: Binding capacity of Mycobind^R relative to a TBM as a ratio *R* was determined using formula (3) as:

R = % binding capacity of RB ÷ % binding capacity of a TBM……. (3)

III. Results

Chemical composition of the test binders

The major minerals contained in the TBM and Mycobind^R are presented in Table 04. These were Muscovite in AC and KC, Kaolinite in CC and MC, Leucite in MC, Microline and Ephicite in VA, Albite and Terranovite in RA, Metanatrolite and Phlogopite in MycobindR.

Table 04. Mineralogical and chemical composition of the TBM and Mycobind^R

TBM and Mycobind^R ID	Prominent Minerals	Chemical formula
AC	Muscovite	$KAl_2(AlSi_3O_{10})(F,OH)_2$
	Hematite-proto	$Fe_{1.9}H_{0.06}O_3$
KC	Quartz	SiO_2
	Muscovite	$KAl_2(AlSi_3O_{10})(F,OH)_2$
	Lizardite	$Mg_3Si_2O_5(OH)_4$
CC	Kaolinite	$Al_2Si_2O_5(OH)_4$
MC	Kaolinite	$Al_2Si_2O_5(OH)_4$
	Leucite	$K[AlSi_2O_6]$
	Lizardite	$Mg_3Si_2O_5(OH)_4$
VA	Pigeonite	$(Ca, Mg, Fe)\ (Mg, Fe)Si_2O_6$
	Microcline	$KAlSi_3O_8$
	Ephesite	$NaLiAl_2\ (Al_2Si_2)O_{10}(OH)_2$
RA	Albite	$NaAlSi_3O_8$ or $Na_{1.0-0.9}Ca_{0.0}$
	Terranovaite	$NaCaAl_3Si_{17}O_{40}8H_2O$
	Sepiolite	$Mg_4Si_6O_{15}(OH)_2{\cdot}6H_2O$
MycobindR	Metanatrolite	$Na_2Al_2Si_3O_{10}$
	Phlogopite	$KMg_3(AlSi_3O_{10})(F,OH)_2$
	Andradite /Melanite	$Ca_3Fe_2(SiO_4)_3$

The elemental-oxide composition of the TBM and Mycobind^R is shown in Table 05. All samples of the TBM and Mycobind^R contained Aluminium and Silicon elements as backbone of the minerals. Other important elements observed as parts of the chemical formula of the prominent minerals in the TBM and Mycobind^R were Iron in AC, VA and MycobindR, Calcium in VA, RA and MycobindR, Potassium in all materials except CC and RA. The VA and Mycobind^R had minerals containing all the main elements Aluminium, Silicon, Iron, Calcium and Potassium.

RA showed the lowest content of Aluminium oxide (alumina) of 0.5%, all the other TBM had content above that of Mycobind^R at 5.1%. Percent Silicon oxide (Silica) contents in CC and RA were above that of Mycobind^R while the TBM had contents from 22-32.8%; a level lower than that of Mycobind^R (49%).

The VA and RA had percent contents of Potassium oxide a little bit higher than that of Mycobind[R]. The VA had Calcium oxide a bit higher than that of Mycobind[R] while the rest of the TBM had percent contents below that of Mycobind[R]. The AC and RA had the highest and the lowest contents of Iron oxide, respectively. Except RA and CC which had lower percent of iron oxide contents, the AC, KC, MC and VA had values above that of Mycobind[R].

The values of CEC for the TBM are also shown in Table 05. The values of CEC for the TBM ranged from 7 meq/100g for CC to 27.2 meq/100g for RA. All the TBM had lower values of CEC compared to that of Mycobind[R] (38.9 meq/100g).

Table 05. The major elemental-oxides found in the binders

TBM/Mycobind[R]	Elemental-oxide composition of the TBM and MycobindR (%)					CEC
	Al_2O_3	SiO_2	K_2O	CaO	Fe_2O_3	(meq/100g)
AC	18.0	26.0	0.22	0.79	45.31	27.2
KC	25.0	31.0	0.01	0.24	39.73	18.8
CC	32.8	61.3	0.63	0.49	2.14	7.0
MC	24.0	34.8	0.52	0.54	36.1	15.4
VA	15.0	22.0	8.78	14.9	26.2	25.4
RA	0.5	75.7	9.54	1.71	0.59	27.2
Mycobind[R]	5.1	49.0	6.99	13.4	19.8	38.9

CEC, Cation exchange capacity

Capacity of the binders to bind aflatoxin

Percent aflatoxin binding capacity for the TBM are presented in Table 06 (across the columns). The percent binding capacity of the TBM ranged from a minimum value of 40 for CC to a maximum value of 85 for RA relative to 98 for the Mycobind[R]. The mean proportions of aflatoxins as adsorbed by the TBM and MycobindR are also shown in Table 6 (across the rows). Proportions of aflatoxins adsorbed were relatively high for AFB1 and AFG1 and low for AFG2 and AFB2.

Table 06. *In-vitro* binding capacity of various clay and ash based materials for aflatoxins

TBM identity	Mean percent of bound individual aflatoxin				Mean percent of total aflatoxin bound	SEM
	AFB1	AFB2	AFG1	AFG2		
AC	97.9	60.6	99.9	32.2	72.6^{ab}	32.5
KC	95.4	40.1	96.1	14.5	61.5^{bc}	40.9
CC	96.6	14.4	31.3	17.3	39.9^{c}	38.5
MC	95.6	32.6	94.6	25.3	62.0^{bc}	38.3
VA	97.9	28.9	71.5	30.7	57.3^{bc}	33.5
RA	94.6	79.8	91.5	72.7	84.7^{ab}	10.2
Mycobind[R]	97.7	99.2	98.8	96.4	98.1^{a}	1.3
Mean	96.5^{a}	50.8^{b}	83.4^{a}	41.3^{b}		
SEM	1.4	30.4	24.9	31.0		

SEM = Standard error of the means; Means with similar superscripts do not differ significantly

The relationship between CEC values of TBM and their elemental-oxides concentration is shown in Figure 01. The relationship presented as correlation coefficients was positive and relatively higher with CaO (0.6), K_2O (0.6) and Fe_2O_3 (0.1) and negative with SiO_2 (-0.1) and Al_2O_3 (-0.9). Similarly, the relationship between percent binding capacity of the TBM and their chemical properties is presented in Figure 02. Their relationship presented as correlation coefficients was positive and relatively higher with values of CEC (0.9), K_2O (0.5), CaO (0.3) and SiO_2 (0.2) and negative with Fe_2O_3 (<-0.1) and Al2O (-0.9).

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

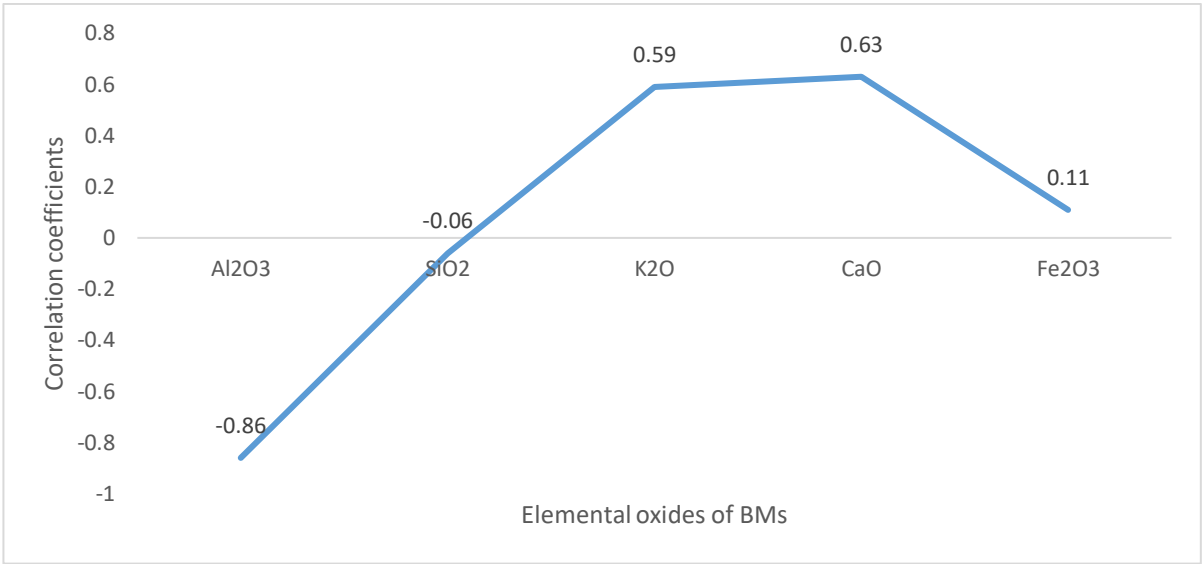

Figure 01. Relationship between values of CEC and the elemental-oxide concentration in TBM.

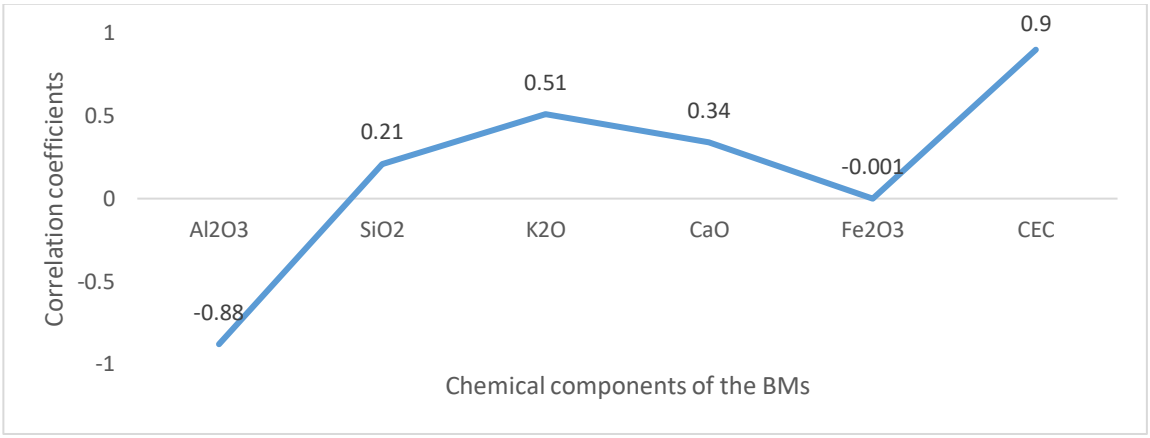

Figure 02. Relationship between percent binding capacity of TBM and their chemical components.

Relationship between the binding capacity and CEC of the TBM with respect to their chemical factors is shown in Figure 03. The relationship presented as correlation coefficient was as high as 0.9.

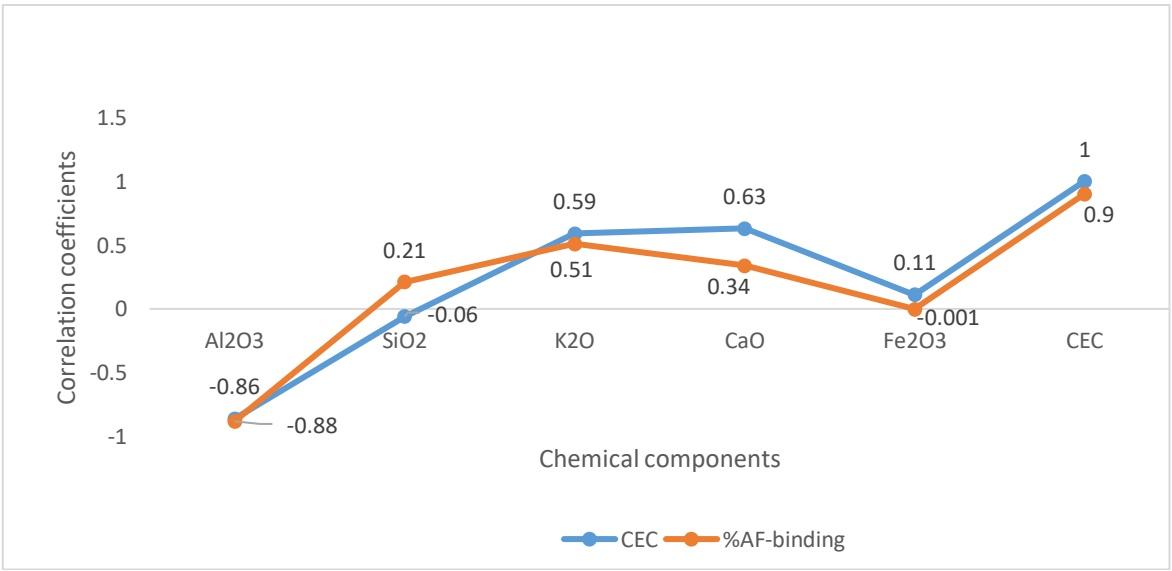

Figure 03. Relationship between the binding capacity, CEC and the concentrations of chemical factors in TBM.

The equivalence of Mycobind^R in binding the total aflatoxins relative to the TBM is shown in Table 07. The binding capacity ratio of Mycobind^R to AC and RA was 1, Mycobind^{R}to KC, MCandVA was 2 and Mycobind^R to CC was 3.

Table 07. Aflatoxin binding capacity ratio of Mycobind^R to that of the TBM

	TBM					
Aflatoxins	AC	KC	CC	MC	VA	RA
AFB1	1.0	1.0	1.0	1.0	1.0	1.0
AFG1	1.0	1.0	3.2	1.0	1.4	1.1
AFB2	1.6	2.5	6.9	3.0	3.4	1.2
AFG2	3.0	6.6	5.6	3.8	3.1	1.3
Overall	1.0	2.0	3.0	2.0	2.0	1.0

IV. Discussion

Among the evaluated binding materials, RA and AC had binding capacity almost equal to that of the reference binder, particularly in binding AFB1 and AFG1, which are the most toxic aflatoxins. Possibly the excellent binding power of these materials was due to their relatively high CEC values. The CEC values of both RA and AC were 27.2 meq/100g of the materials and are equivalent to the CEC value for the reference material. High CEC values of many binding materials have been reported to promote their capacity to bind aflatoxins (Vikiru *et al.* 2015). The relatively high values of Calcium (Ca^{2+}) and Potassium (K^{+}) contents in the alumino-silicate minerals of the evaluated materials seemed to promote values of CEC of the materials. Studies have shown that concentrations of Ca^{2+} and K^{+} ions make a great contribution to CEC levels in alumino-silicate materials (Rayment and Higginson, 1992). Presence of Silicon (Si^{4+}), Aluminium (Al^{3+}) and Iron (Fe^{3+}) seemed to have low or negative influence on the CEC values of the TBM/Mycobind^R. According to Brady and Weil (1990), values of CEC increase with decreasing acidity and vice versa. The ions Si^{4+}, Al^{3+} and Fe^{3+} are acidity promoter unlike Ca^{2+} and K^{+} (Brady and Weil, 1990), hence negatively influencing CEC values of the TBM/Mycobind^R and subsequently their capacity to bind aflatoxins in solution. Disregarding other factors such as structural effect of a material, probably materials like CC showed low capacity for aflatoxins binding partly due to its higher concentration of Al^{3+} and Si^{4+}, and partly due to its relatively higher content of Kaolinite type of mineral, which has low CEC (Brown and Lemon, 2016). Furthermore, KC and MC could not bind aflatoxins efficiently possibly due to relatively higher concentration of Al^{3+} and Fe^{3+}.

The X-RD analysis showed presence in the TBM, of prominent mineral components that can influence aflatoxin binding. The results showed that just like the Mycobind^R, RA and AC contained major minerals such as Andranite/Melanite, Terranovite and Albite; all of which contain Calcium and Phlogopite as well as Muscovite which contain Potassium. Possibly these components rendered RA and AC relatively superior to others in binding aflatoxins. Kang *et al.* (2016) suggested that in aflatoxin binding, ions Ca^{2+} in particular, synchronously bonds to two aflatoxin carbonyls and at the same time binds to the four oxygen atoms of the Si-O ring on the clay binder surface. However, AC had low Ca^{2+} and K^{+} cations yet its CEC value was relatively high enough to favour high aflatoxin binding capacity. Seemingly, the way active cations such as Calcium and Potassium are incorporated in different structures of the TBM and their associations with other structural elements may affect adsorptive potential of the TBM.

Generally, the chemical composition values for the TBM evaluated in this study were within value ranges reported for alumino-silicate based materials studied for various purposes including as feed additives. The general alumina content in the materials was within the range reported in other studies of 0.45-21.7% (Karnland, 2010) and 13.2% (Massinga *et al.* 2010) cited by Anjos *et al.* (2016) except for CC which contained higher level of alumina at about 33%. Except for RA which showed much higher percent content of silica, the other TBM had content similar to the reported values for clay materials ranging from 1.1-69.0% (mean of 59.6%) (Karnland, 2010) and 44.3-71.0% (mean of 55.3%) (Mukasa-Tebandeke *et al.* 2015). Just like Mycobind^R, VA and RA had content of Potassium oxide above the previously reported range of 0.1-3.3% (Karnland, 2010) and 0.1-2.6% (Mukasa-Tebandeke *et al.* 2015) and 0.1% (Massinga et al. 2010) cited by Anjos *et al.* 2016) for high aflatoxin binding. Content of

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

Potassium oxide of 0.01% in KC was below the reported levels. Contents of Calcium oxide in all TBM were found within the previously reported range of 0.1-31.4% (Karnland, 2010; Mukasa-Tebandeke *et al.* 2015 and Massinga *et al.* 2010) cited by Anjos *et al.* 2016)) for binders. Except the CC and RA, the rest of the TBM showed content of Iron oxide above the previously reported range of 0.2-14.8% Karnland, 2010; Mukasa-Tebandeke *et al.* 2015 and Massinga *et al.* 2010) cited by Anjos *et al.* 2016) for binders. From the comparative composition of the TBM it seems that the materials do not differ from other materials of alumino-silicate nature including those proved to bind aflatoxins.

Alumino-silicates based materials have been reported to have CEC (meq/100g) values ranging from 10 (Kaolinite mineral) to 100 (Illite and Smectite minerals) and medium values are found around value of 25 (Brown and Lemon, 2016). Except for the CC that showed low value of 7 meq/100g, the rest of the TBM had CEC values within the documented range as were observed from 15.4meq/100g (MC) to 38.9 meq/100g for MycobindR.

The results for aflatoxin binding capacity of the TBM concurred with results of other previous related in-vitro studies in which binding capacity levels of clay-based binders such as bentonites (about 90%)has been reported (Manafi *et al.* 2009; Kong *et al.* 2014). The Mycobind^{R} employed as a reference in this study missed manufacturer's information displaying its capacity to bind aflatoxins. However, in our analysis we found that it could bind about 98% of the total aflatoxins subjected to it. A similar product Agrolite-Mycobind^{R} evaluated in Kenya showed aflatoxin binding capacity of 95% (www.pasitokenyaltd.com). Regarding minimum experimental set-up standards, though slightly higher, the binding capacity of 98% observed for the Mycobind^{R} in this study matched closely to 95% value reported for the Agrolite-Mycobind^{R}.

The binding capacity ratio of Mycobind^{R} to TBM observed in this study, conversably implied that AC and RA bind 100%, KC, MC and VA bind 50% and CC binds 33.3% of the total aflatoxins in solution. This indicates though in varying levels, the locally available crude materials (clay and ash based resources) have potential to adsorb aflatoxins in solution media and possibly can reduce aflatoxin contamination of feeds.

The AFB1 and AFG1 were highly adsorbed into the TBM as compared to AFB2 and AFG2. Probably this is because compared to AFB2 and AFG2, the AFB1 and AFG1 have higher polarity of the β-dicarbonyl group which is a key functional group of the aflatoxins (Grant and Phillips, 1998). With the polarity respect, AFB1 was rendered the most adsorbed by the TBM followed by AFG1. This is advantageous since the toxicity of the aflatoxins tends to follow this order of reactivity, which was also obeyed by our results in this study. The aflatoxin binding capacity of the evaluated materials (especially RA and AC) can be confirmed on *in-vivo* test where the dietary and animal's GIT factors are automatically accommodated. However, since exported binders are costly to farmers in low-income countries, occasionally the material can be used in feeds to reduce hazard effects of aflatoxins to animals. In addition, traditionally farmers have been using an array of such materials for various intentions including uses in animal feeds. It has been observed that wild animals and birds are less affected by many natural toxins probably including aflatoxins due to their instincts related to geophagia (Diamond, 1999 and Mahaney and Krishnamani, 2003). Harnessing this natural phenomenon may be economically helpful to poor farmers as one of strategies in lowering aflatoxin menace which is difficult to avoid in feeds.

IV. Conclusion

The test materials we evaluated in the study had varying capacity levels of binding aflatoxins in solution. The crude materials AC (Arusha clay) and RA (Rice-husk ash) have the relatively higher potential to bind aflatoxins equivalent to the commercial product Mycobind^{R} employed in the study for reference purpose. Since traditionally these cheap materials are used for various purposes in animals, occasionally they could be utilized to minimize exposure of aflatoxin load to animals through contaminated feeds. Further studies are recommended to test binding capacity of these materials in refined form and when used in combinations of two or more of them, using both on in-vitro and in-vivo trials.

Conflict of interests

The authors declare that there is no conflict of interest related to this study.

Acknowledgements

We would like to acknowledge the Institute of Rural development Planning (IRDP)-Dodoma, which funded this study. We are also grateful to the Department of Geology and Mineral Resources of the University of Dar es Salaam (UD) and Tanzania Food and Drugs Authority (TFDA) for their facilitation in the sample analysis and provision of the premises for the experiments.

V. References

[1]. Andretta, I., Kipper, M., Lehnen, C. R., Hauschild, L., Vale, M. M. and Lovatto, P. A. (2012). Meta analytical study of productive and nutritional interactions of mycotoxins in growing pigs. Animal, 6(9), 1476-1482. https://doi.org/10.1017/S1751731111002278

[2]. Anjos, F. D., Ledoux, D. R., Rottinghaus, G. E. and Chimonyo, M. (2016). Efficacy of Mozambican bentonite and diatomaceous earth in reducing the toxic effects of aflatoxins in chicks. World Mycotoxin Journal, 9(1), 63-72. https://doi.org/10.3920/WMJ2014.1842

[3]. Arapcheska, M., Jovanovska, V., Jankuloski, Z., Musliu, Z. and Uzunov, R. (2015). Impact of aflatoxins on animal and human health. Int. J. Innov. Sci. Eng. Technol, 2(2), 156-161.

[4]. Brady, N. C. and Weil, R. R. (1990). The nature and properties of soils. Macmillan, New York. The nature and properties of soils. 10th ed. Macmillan, New York. p. 201.

[5]. Bremner, J. M. (1960). Determination of nitrogen in soil by the Kjeldahl method. The Journal of Agricultural Science, 55(1), 11-33. https://doi.org/10.1017/S0021859600021572

[6]. Brown, K. and Lemon, J. (2016). Fact Sheets Cations and Cation Exchange Capacity. Retrieved from *http://www.soilquality.org.au/factsheets/cation-exchange-capacity.*

[7]. Devreese, M. (2013). Development of in vitro and in vivo models for testing the efficacy of mycotoxin detoxifying agents and their possible interaction with oral absorption of veterinary drugs (Doctoral dissertation, Ghent University).

[8]. Diamond, J. M. (1999). Evolutionary biology: dirty eating for healthy living. Nature, 400(6740), 120. https://doi.org/10.1038/22014

[9]. Diaz, D. E., Hagler, W. M., Hopkins, B. A. and Whitlow, L. W. (2003). Aflatoxin binders I: in vitro binding assay for aflatoxin B1 by several potential sequestering agents. Mycopathologia, 156(3), 223-226. https://doi.org/10.1023/A:1023388321713

[10]. Diaz, D. E. and Smith, T. K. (2005). Mycotoxin sequestering agents: practical tools for the neutralization of mycotoxins.

[11]. Dhanasekaran, D., Shanmugapriya, S., Thajuddin, N. and Panneerselvam, A. (2011). Aflatoxins and aflatoxicosis in human and animals. In *Aflatoxins-Biochemistry and Molecular Biology*. In Tech. https://doi.org/10.5772/22717

[12]. Feddern, V., Dors, G. C., Tavernari, F. D. C., Mazzuco, H., Cunha, A., Krabbe, E. L. and Scheuermann, G. N. (2013). Aflatoxins importance on animal nutrition. In *Aflatoxins-Recent Advances and Future Prospects.* InTech. https://doi.org/10.5772/51952

[13]. Grace, D. (2013). Animals and aflatoxins [No. 20 (5)]. International Food Policy Research Institute (IFPRI).

[14]. Grant, P. G. and Phillips, T. D. (1998). Isothermal adsorption of aflatoxin B1 on HSCAS clay. Journal of agricultural and food chemistry, 46(2), 599-605. https://doi.org/10.1021/jf970604v

[15]. Habeeb, G. A. and Mahmud, H. B. (2010). Study on properties of rice husk ash and its use as cement replacement material. Materials research, 13(2), 185-190. https://doi.org/10.1590/S1516-14392010000200011

[16]. Jen, J. J. S. and Chen, J. (Eds.). (2017). Food safety in China: Science, technology, management and regulation. John Wiley & Sons. https://doi.org/10.1002/9781119238102

[17]. Kang, F., Ge, Y., Hu, X., Goikavi, C., Waigi, M. G., Gao, Y. and Ling, W. (2016). Understanding the sorption mechanisms of aflatoxin B1 to kaolinite, illite, and smectite clays via a comparative computational study. Journal of hazardous materials, 320, 80-87. https://doi.org/10.1016/j.jhazmat.2016.08.006

Published with open access at journalbinet.com
EISSN: 2414-5009, © 2018 The Authors, Research Paper

[18]. Kannewischer, I., Arvide, M. G. T., White, G. N. and Dixon, J. B. (2006). Smectite clays as adsorbents of aflatoxin B1: initial steps. Clay Science, 12 (Supplement2), 199-204.
[19]. Karnland, O. (2010). Chemical and mineralogical characterization of the bentonite buffer for the acceptance control procedure in a KBS-3 repository (No. SKB-TR--10-60). Swedish Nuclear Fuel and Waste Management Co.
[20]. Khan, M. Z., Hameed, M. R., Hussain, T., Khan, A., Javed, I., Ahmad, I., Hussain, A., Saleemi, M. K. and Islam, N. U., 2013. Aflatoxin residues in tissues of healthy and sick broiler birds at market age in Pakistan: A one-year study. Pak Vet J, 33(4), 423-427.
[21]. Kolosova, A. and Stroka, J. (2012). Evaluation of the effect of mycotoxin binders in animal feed on the analytical performance of standardised methods for the determination of mycotoxins in feed. Food Additives & Contaminants: Part A, 29(12), 1959-1971. https://doi.org/10.1080/19440049.2012.720035
[22]. Kong, C., Shin, S. Y. and Kim, B. G. (2014). Evaluation of mycotoxin sequestering agents for aflatoxin and deoxynivalenol: an in vitro approach. Springer Plus, 3(1), 346. https://doi.org/10.1186/2193-1801-3-346
[23]. Korotkova, T. G., Ksandopulo, S. J., Donenko, A. P., Bushumov, S. A. and Danilchenko, A. S. (2016). Physical Properties and Chemical Composition of the Rice Husk and Dust. Oriental Journal of Chemistry, 32(6), 3213-3219. https://doi.org/10.13005/ojc/320644
[24]. Lopez, C., Ramos, L., Bulacio, L., Ramadan, S. and Rodriguez, F. (2002). Aflatoxin B1 content in patients with hepatic diseases. MEDICINA-BUENOS AIRES-, 62(4), pp.313-316.
[25]. Mahaney, W. C. and Krishnamani, R. (2003). Understanding geogphagy in animals: standard procedures for sampling soils. Journal of Chemical Ecology, 29(7), 1503-1523. https://doi.org/10.1023/A:1024263627606
[26]. Manafi, M., Narayanaswamy, H. D. and Pirany, N. (2009). In vitro binding ability of mycotoxin binder in commercial broiler feed. African Journal of Agricultural Research, 4(2), 141-143.
[27]. Mohamed, R. M., Mkhalid, I. A. and Barakat, M. A. (2015). Rice husk ash as a renewable source for the production of zeolite NaY and its characterization. Arabian Journal of Chemistry, 8(1), 48-53. https://doi.org/10.1016/j.arabjc.2012.12.013
[28]. Massinga Jr, P. H., Focke, W. W., De Vaal, P. L. and Atanasova, M. (2010). Alkyl ammonium intercalation of Mozambican bentonite. Applied Clay Science, 49(3), 142-148. https://doi.org/10.1016/j.clay.2010.04.017
[29]. Mok, C. H, Shin S. Y. and Kim B. G. (2013). Aflatoxin, deoxynivalenol, and zearalenone in swine diets: Predictions on growth performance. Revister Colomb Cienc Pecu, 26, 243-254.
[30]. Moreno-Pedraza, A., Valdés-Santiago, L., Hernández-Valadez, L. J., Rodríguez-Sixtos Higuera, A., Winkler, R. and Guzmán-de Peña, D. L. (2015). Reduction of aflatoxin B1 during tortilla production and identification of degradation by-products by direct-injection electrospray mass spectrometry (DIESI-MS). salud pública de méxico, 57, 50-57. https://doi.org/10.21149/spm.v57i1.7402
[31]. Mukasa-Tebandeke, I. Z., Ssebuwufu, P. J. M., Nyanzi, S. A., Schumann, A., Nyakairu, G. W. A., Ntale, M. and Lugolobi, F. (2015). The elemental, mineralogical, IR, DTA and XRD analyses characterized clays and clay minerals of Central and Eastern Uganda.
[32]. Rayment, G. E. and Higginson, F. R. (1992). *Australian laboratory handbook of soil and water chemical methods*. Inkata Press Pty Ltd.
[33]. Phillips, T. D., Sarr, A. B. and Grant, P. G. (1995). Selective chemisorption and detoxification of aflatoxins by phyllosilicate clay. Natural toxins, 3(4), 204-213. https://doi.org/10.1002/nt.2620030407
[34]. Phillips, T. D., Lemke, S. L. and Grant, P. G. (2002). Characterization of clay-based enter sorbents for the prevention of aflatoxicosis. In: Mycotoxins and food safety. Springer, Boston, MA. pp. 157-171. https://doi.org/10.1007/978-1-4615-0629-4_16
[35]. SAS Institute Inc. (2004). SAS/STAT® 9.2 user's guide. *(Ver. 9.1)*.
[36]. Sassahara, M., Netto, D. P. and Yanaka, E. K. (2005). Aflatoxin occurrence in foodstuff supplied to dairy cattle and aflatoxin M1 in raw milk in the North of Parana state. Food and chemical toxicology, 43(6), 981-984. https://doi.org/10.1016/j.fct.2005.02.003
[37]. Vekiru, E., Fruhauf, S., Rodrigues, I., Ottner, F., Krska, R., Schatzmayr, G. and Bermudez, A. J. (2015). In vitro binding assessment and in vivo efficacy of several adsorbents against aflatoxin B1. World Mycotoxin Journal, 8(4), 477-488. https://doi.org/10.3920/WMJ2014.1800