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Modified Natural Clinoptilolite Detoxifies Small Mammal's Organism Loaded with Lead I. Lead Disposition and Kinetic Model for Lead Bioaccumulation

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Abstract Zeolites, especially clinoptilolites, have wide application in removing heavy metals from different solutions and wastewater. The detoxification capacity of the clinoptilolite sorbent KLS–10-MA, a modified natural Bulgarian zeolite, applied as a food supplement in conditions of an ecotoxicological experiment with conventional food and lead was demonstrated for the first time. Laboratory mice, inbred imprinting control region strain, were used in a 90-day ecotoxicological experiment. Animals were divided into four experimental groups. Lead bioaccumulations in exposed and non-supplemented/supplemented with KLS–10-MA animals were compared. As additional control, healthy animals non-exposed to Pb were fed with conventional forage mixed with 12.5% KLS–10-MA. The dietary inclusion of the sorbent reduced Pb concentrations in exposed and supplemented mice by 84%, 89%, 91%, 77%, and 88% in carcass, liver, kidneys, bones, and feces, respectively. A mathematical model was proposed to outline the common trends of bone Pb bioaccumulation in exposed and non-supplemented/supplemented animals. Characteristic parameters of the kinetics of Pb concentrations were determined. Based on the model, the coefficient of absorption of Pb by gastrointestinal mucosa in the supplemented mice was found—η=3.53% (versus η=15% in non-supplemented ones). The present study clearly indicates that there is a realistic perspective to create a new drug based on modified natural clinoptilolites in cases of chronic heavy metal intoxication, without negatively affecting the environment.

Keywords Lead bioaccumulation · Clinoptilolite · Detoxification · Laboratory mice · Mathematical model · Carcass · Liver · Kidneys · Bone · Feces

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Introduction

Lead (Pb) is one of the most popular environmental pollutants resulting from anthropogenic activities. It is a highly deleterious heavy metal for organisms, causing alterations in growth and behavior, renal function deficits, hypertension, osteoporosis, lead-induced anemia, etc. [1–3]. Lead is also a significant genotoxic agent [4–6]. Therefore, the possibilities of lead neutralization are of great importance.

A significant part of lead (Pb^{2+}) intake is possible to be neutralized in the stomach. For that purpose, zeolites, which reveal a unique selective adsorption, are considered as a reliable means [7].

Clinoptilolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, consisting of three-dimensional frameworks of SiO_4^{4-} and AlO_4^{5-} tetrahedra linked through the shared oxygen atoms [8]. They are the most abundant natural zeolites [9], occurring in volcanic and sedimentary rocks [10, 11]. Their molar Si/Al ratio is above 4 [12, 13]. Clinoptilolites have a relatively open structure with a total pore volume of approximately 35% [14], and chemical formula (K, Na, Ca, Mg).$(AlSi_{43}O_8)$.$5H_2O$ [15].

The structure of clinoptilolites is characterized by large intersecting open channels of ten- and eight-member tetrahedral rings [7]. Such structure ensures the clinoptilolite capacity of to absorb and accumulate heavy metals. Therefore, clinoptilolites are often utilized to reduce them from solutions [16–20].

Why are clinoptilolites a perfect heavy metal-trap? The Si-block is neutral, while the Al-block in crystalline unit is negative, thus it charges the mineral's lattice negatively. The existence of Na, K, and/or Ca cations determines the neutrality of the minerals. These cations are exchanged in solutions with cations of certain metals, such as Pb, Cd, Hg, etc. [9, 21].

In order to meet increasingly stringent Environmental Quality Criteria, clinoptilolites are widely applied for removing heavy metals from polluted streams and wastewater [16, 21–27] and for the elimination of gas pollutants in confinement facilities [11, 28]. Besides, this mineral has widespread applications in agriculture [10, 15, 29]. According to EMFEMA (2005), zeolites also allow better performance of intestinal microflora [30].

This work presents sorbet's (KLS–10-MA) features in the context of a possible effective use of the mineral as a reliable tool for detoxification of animal organisms chronically poisoned by heavy metals, particularly lead.

There is a lack of data regarding the behavior and adsorption capacity of this mineral modification in animal organisms. The process of ions exchange can occur in the gastrointestinal tract, and thus the resorption of toxic metals through the intestinal mucosa could be prevented in a great extent. The investigation is targeted to study the degree of the positive effect of a modified clinoptilolite KLS–10-MA (water modification), applied as food-additive on Pb bioaccumulation in laboratory inbred imprinting control region (ICR) line mice. In addition, a mathematical model of Pb bioaccumulation in exposed and exposed–supplemented animals was proposed. In a separate article, evidences that clinoptilolite is practically non-toxic are presented [31]. As experimental animals, laboratory mice of inbred line ICR were chosen.

Materials and Methods

The ecotoxicological experiment was conducted according to approved protocols and in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations.

The ecotoxicological experiment covered 90 days. Only males, about 8 weeks of age, laboratory mice, inbred ICR strain, were used. The exposure to Pb was performed as the mice were treated with 0.05 N solution of lead nitrate diluted 1:10 in the drinking water during all the investigating period. The clinosorbent KLS–10-MA in form of powder was mechanically mixed to a 12.5% concentration with conventional granulated forage for small rodents.

Animals were arranged in four groups each of 60 specimens, as follows: group 1, (control) animals fed with conventional food for small rodents and water; group 2 animals fed with conventional food + clinosorbent KLS–10-MA and water; group 3 animals fed with conventional food and water + $Pb(NO_3)_2$; group 4 animals fed with conventional food + KLS–10-MAand water + $Pb(NO_3)_2$.

Two variants of the feed mixture were prepared: (1) standard (conventional food) and (2) standard food treated with 12.5% sorbent KLS–10-MA. The elements' composition in the variants 1 and 2 are presented in Table 1.

The modified clinoptilolite was prepared by Nikolay Popov through a treatment of natural clinoptilolite (zeolite containing 82% clinoptilolite) obtained from the region of East Rhodopes in South Bulgaria (Fig. 1). The natural clinoptilolite was heat-treated at 240–250°C and then chemically and mechanically activated with 10% alkaline salt, addition of 25 wt.% distilled water, and 4 h processing in ball-crusher (wet activation). Chemical composition of the clinoptilolite sorbent KLS–10-MA was determined by common analytical method for silicate materials. Cations exchange capacity was determined according to the method of Ming ad Dixon [32].

Experimental animals were bread in vivarium and housed in individually ventilated cages. The physical size of the cages was in accordance with European standards. The bedding material was obtained from an ISO-2000-accredited supplier. Mice were acclimatized for a 7-day period before starting the experiment. A standard temperature of between 19°C and 23°C, a humidity of 45–60%, and a 12-h light/night cycle were kept all the time. The food was

Table 1 Quantities of some elements (milligrams per kilogram) in the two variant feed mixtures of ICR mice

Elements	Na	K	Ca	Mg	Fe	Zn	Cu	Mn	Pb
Standard food	885±45	8,164±245	7,575±227	2,319±92	412.5±78	76.99±15.4	20.9±1.4	101.9±10.2	2.25±0.3
Food+12.5% KLS–10-MA	1,924±96	9,397±282	8,415±252	2,205±88	378.6±72	75.22±14.9	15.4±0.9	121.2±11.9	2.76±0.3

in the form of pellets and not withheld at any time during the experiment. All mice were allowed access to food and water ad libitum. The water, food, and bedding material were daily inspected and changed when necessary. The animals were neither medicated nor vaccinated.

The concentrations of Pb in the whole body, liver, kidney, bones, and feces of the control and experimental animals were determined on days 15, 40, 60, and 90 from the beginning of the experiment.

To determine the Pb concentration, after the removal of the alimentary tract, the tissues and some internal organs were oven dried at 60 C to a constant weight. The dried tissues were dissolved in a mixture of concentrated nitric–perhloric acid (4:1) [33]. The concentrations of Pb and the element composition of the two food variants were determined in a certified laboratory by atomic emission spectrometry with inductively coupled plasma on a GFAAS–Varian instrument. The detection limits were 0.002 mg/l for Mn; 0.004 mg/l for Cd; 0.005 mg/l for Zn; 0.03 mg/l for Pb; 0.04 mg/l for Fe; and 0.5 mg/l for Ca, K, Mg, and Na.

Statistical analysis was done by using the SPSS Package for Windows, version 15.0. Differences were considered to be significant when p values were lower than 0.05 ($p<0.05$). First, the data were processed according to the Kolmogorov–Smirnov test for normality in each group. All groups showed normal distributions, and the data were then analyzed by analysis of variance and subsequent Tukey high statistical difference test and Dunnet test for estimating individual differences.

Mathematical Model

The present mathematical model is confined to Pb bioaccumulation in animals' bones. Three "compartments" of Pb movement are considered: gastrointestinal tract, blood, and bones (Fig. 2). One can assume that Pb is distributed evenly into compartments, which allows the use of differential equation for its kinetics. After entering in gastrointestinal tract, Pb moves to blood, and then to bones. Thus, the following system of ordinary differential equations takes place:

$$\frac{dx}{dt} = -a_1x - a_2x \quad (1)$$

$$\frac{dy}{dt} = a_1x - a_3y - a_4y \quad (2)$$

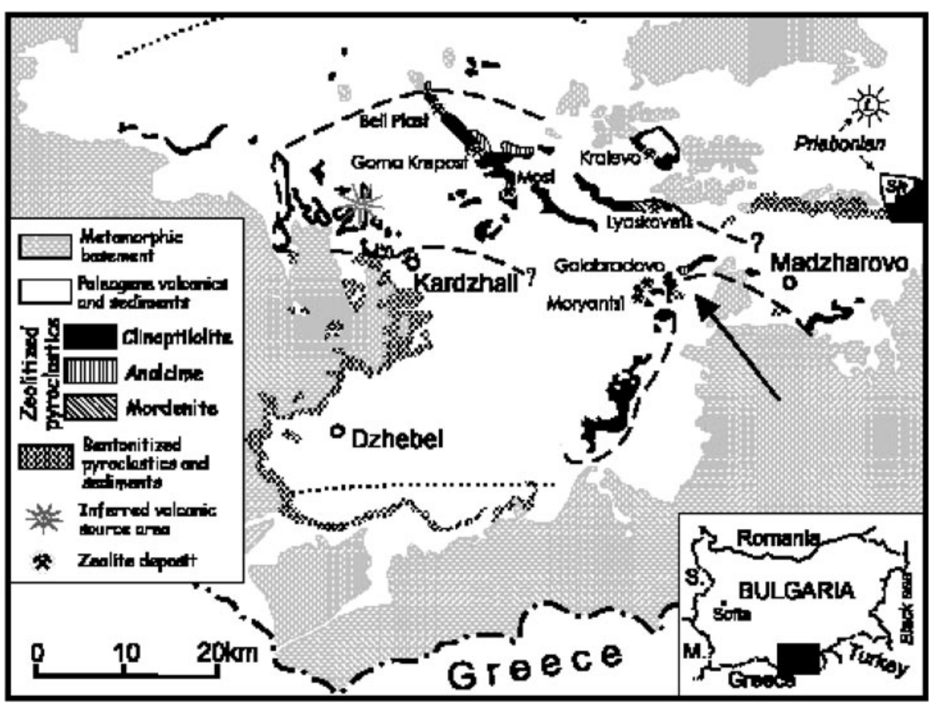

Fig. 1 A map of the distribution of natural clinoptilolites in Bulgaria. The *arrow* indicates Golobradovo deposit

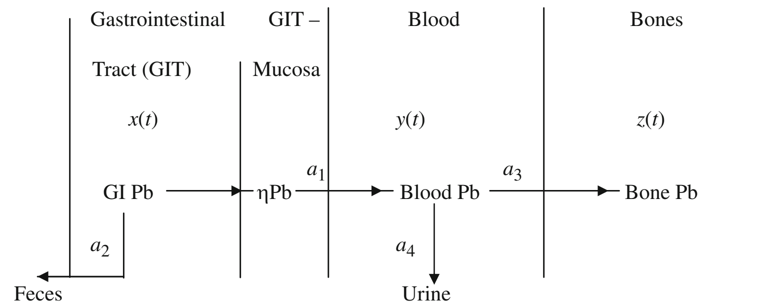

Fig. 2 Scheme of the compartments, pathways, and rate constants. $x(t)$, $y(t)$, and $z(t)$ are the Pb concentrations [milligrams per kilogram] (varied with the time t) in the gastrointestinal tract, blood, and bones, respectively

$$\frac{dz}{dt} = a_3 y \tag{3}$$

Under initial conditions:

$$t_0 = 0,\ x(t_0) = x_0 = A,\ y(t_0) = 0,\ z(t_0) = z_0 \tag{4}$$

where x, y, and z are the concentrations [milligrams per kilogram] of Pb in the gastrointestinal tract, blood, and bones, respectively; t is time [days]; t_0 is the moment when the experiment starts; a_1 ($[a_1]$=[day^{-1}]) and a_3 ($[a_3]$=[day^{-1}]) are the rate constants of Pb accumulation in blood and bones, respectively; a_2 ($[a_2]$=[day^{-1}]) and a_4 ($[a_4]$=[day^{-1}]) are the rate constants of Pb excretion through the feces and urine, respectively; dx/dt, dy/dt, and dz/dt are the rates of change in Pb levels in the three compartments, respectively.

Results

Experimental Results

The chemical composition of the natural Bulgarian clinoptilolite from Golobradovo and modified clinoptilolite KLS–10-MA are presented in Table 2. The values of exchangeable alkaline and alkaline earth cations are displayed in Table 3.

The concentration of Pb [milligrams per kilogram] in the whole body, liver, and kidneys measured in mice from groups 3 and 4 are presented in Fig. 3. The concentration of Pb in bones and feces are displayed in Figs. 4 and 5, respectively. Point "0" of the time axis in all figures corresponds to the concentration measured in the control group.

The highest Pb concentrations were established in feces (Fig. 5), followed by those in bones (Fig. 4) of the mice from group 3. The background Pb level in carcass, liver, kidney, bones, and feces of the control mice were 0.22±0.06, 0.5±0.07, 0.44±0.08, and 23.6±6.7 mg/kg, respectively. On day 90, the Pb concentrations in group 3 in carcass, liver, kidney, bones, and feces were 1,467-fold, 133-fold, 1,337-fold, 1,523-fold, and 406-fold higher compared with those in the control group. Regarding group 4, these Pb concentrations were 237-fold, 17-fold, 125-fold, 357-fold, and 51-fold higher compared with the control group. Significant differences ($p>$ 0.001) were established in carcass, kidney, bone, and feces between groups 3 and 4 after day 45. The reduction in Pb levels in the exposed and supplemented mice compared with exposed unsupplemented ones was as follows—84%, 89%, 91%, 77%, and 88% for carcass, liver, kidney, bones, and feces, respectively.

Significant differences ($p<0.001$) were established between Pb bioaccumulations in carcass, kidney, and bones at the time points 15, 45, 60, and 90 and between Pb contents in feces at the time points 15, 45, and 60 in group 3. The following ratios Pb_{90}/Pb_{15} were calculated for carcass, liver, kidney, bones, and feces—in group 3, 7.68, 7.89, 3.85, 7.43, and 2.85 and in group 4, 2.83, 0.7, 1.02, 4.27, 1.3, respectively (Fig. 6).

When denote $(Pb_{90}/Pb_{15})_{\mathrm{Group\ 3}}=R_3$ and $(Pb_{90}/Pb_{15})_{\mathrm{Group\ 4}}=R_4$, the following relations take place:

$$(R_3/R_4)_{\mathrm{Carcass}} = 2.7; (R_3/R_4)_{\mathrm{Feces}} = 2.19 \tag{5}$$

Table 2 Chemical composition (%) of clinoptilolite samples: natural (Golobradovo, South-East Rhodops Mountain, Bulgaria) and modified (KLS–10-MA)

Clinoptilolite chemical composition, %		
Constituent	Natural	KLS–10-MA
SiO_2	69.3	66.09
Al_2O_3	11.6	10.62
Fe_2O_3	1.7	0.76
TiO_2	0.1	0.12
CaO	2.11	3.03
MgO	2.37	0.33
Na_2O	0.98	4.49
K_2O	1.59	3.11
I. L.	10.32	11.37
Molar Si/Al	5.97	6.22

Table 3 Cation exchange capacity (milliequivalent per 100 g, NH_4^+) and exchangeable cations (milliequivalent per 100 g) of clinoptilolite samples from Golobradovo, South-East Rhodopes Mountain, Bulgaria (natural and modified)

Samples	Natural	KLS–10-MA
Cation exchange capacity	102	139.5
Exchangeable cations		
Na^+	23.34	133.14
K^+	19.14	36.08
Ca^+	61.22	24.68
Mg^+	1.05	2.41
Total	104.75	196.31

$$(R_3/R_4)_{\text{Liver}} = 11.27; (R_3/R_4)_{\text{Kidney}} = 3.77; (R_3/R_4)_{\text{Bone}} = 1.74 \quad (6)$$

To consider the bioaccumulation differences in the different tissues resulting from the sorbent supplementation more accurately, the following ratios were calculated for day 90:

$$\text{Group 3} \quad \text{Pb}_{\text{Bone}}/\text{Pb}_{\text{Liver}} = 19;\ \text{Pb}_{\text{Bone}}/\text{Pb}_{\text{Kidney}} = 2.5;\ \text{Pb}_{\text{Kidney}}/\text{Pb}_{\text{Liver}} = 7.6 \quad (7a)$$

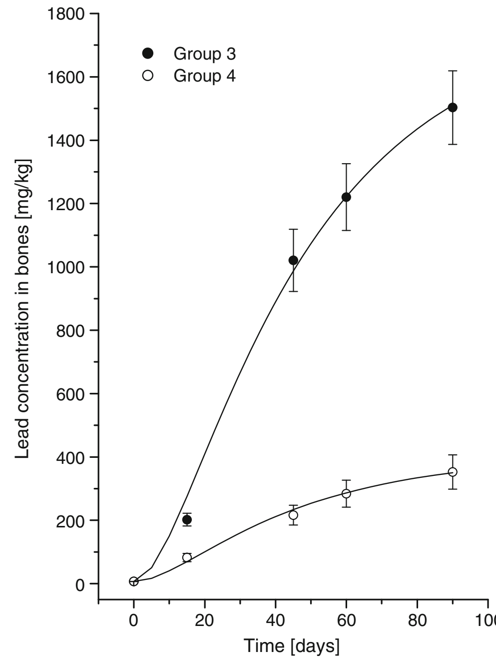

Fig. 4 Lead concentration in bones of ICR mice from groups 3 and 4 during the ecotoxicological experiment—model solutions and experimental points. Time point "*0*" corresponds to the concentrations in the control group

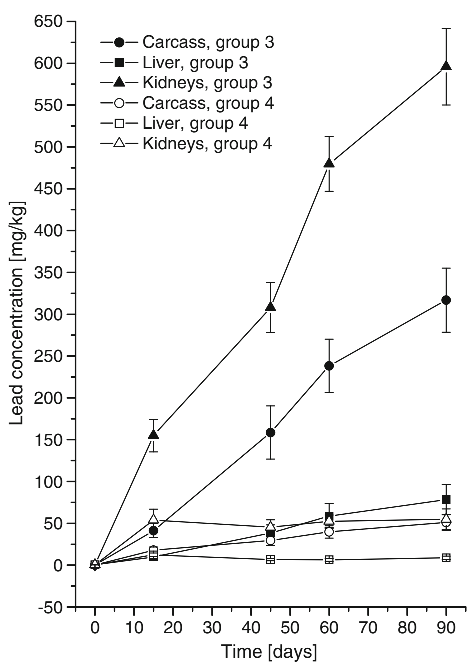

Fig. 3 Lead concentrations in carcass, liver, and kidneys of ICR mice from groups 3 and 4 during the ecotoxicological experiment. Time point "*0*" corresponds to the concentrations in the control group

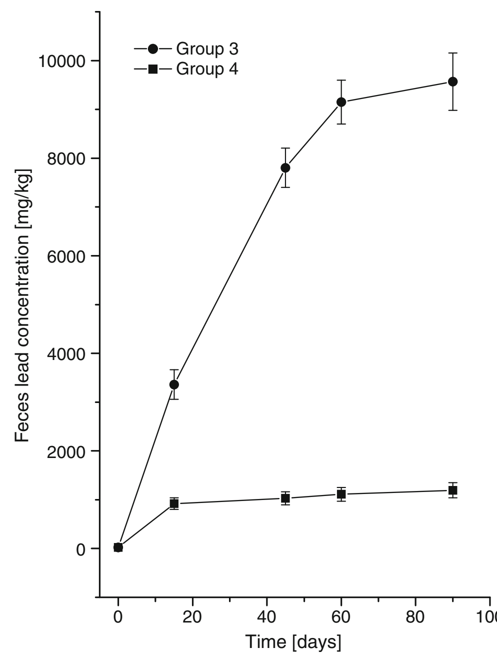

Fig. 5 Lead concentration in feces of ICR mice from groups 3 and 4 during the ecotoxicological experiment. Time point "*0*" corresponds to the concentrations in the control group

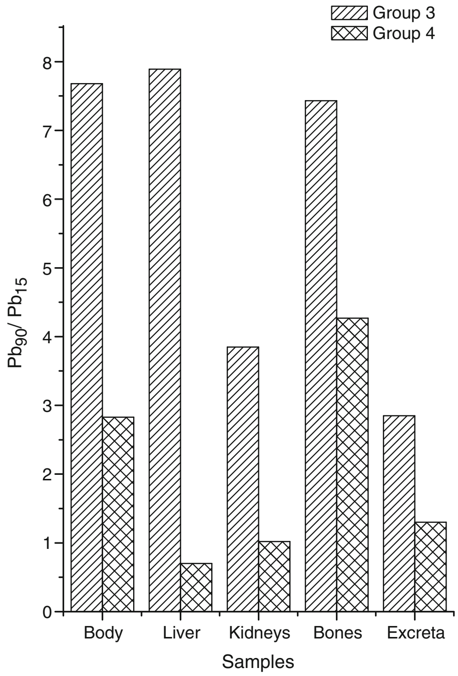

Fig. 6 The Pb_{90}/Pb_{15} ratio for carcass, liver, kidney, bones, and feces, indicating the significant reduction of Pb bioaccumulation in group 4 versus group 3 due to the clinoptilolite sorbent supplement

$$\text{Group 4} \quad Pb_{Bone}/Pb_{Liver} = 40;\ Pb_{Bone}/Pb_{Kidney} = 6.4;\ Pb_{Kidney}/Pb_{Liver} = 6.3 \tag{7b}$$

Another ratio, calculated also for day 90, could help to estimate the significant decreasing of Pb bioaccumulation in the conditions of the sorbent supplementation:

$$\text{Bone} \quad Pb_{Group3}/Pb_{Group4} = 4.3 \tag{8a}$$

$$\text{Liver} \quad Pb_{Group3}/Pb_{Group4} = 9 \tag{8b}$$

$$\text{Kidney} \quad Pb_{Group3}/Pb_{Group4} = 11 \tag{8c}$$

Modeling Results

For the Eq. 3 under conditions (4), the following analytical solution was obtained:

$$z(t) = z_0 + Aa_1a^3\left(\frac{1}{b_1b_2} - \frac{1}{b_1(b_2-b_1)}e^{-b_1t} + \frac{1}{b_2(b_2-b_1)}e^{-b_2t}\right) \tag{9}$$

where $b_1=a_1+a_2$ and $b_2=a_3+a_4$.

The solution $z(t)$ represents the process of Pb bioaccumulation in the bones of the mice. Graphical time course of bone Pb is present in Fig. 4. The initial condition $z(t_0)=z_0$ corresponds to the bone Pb concentration in the control group, $z_0=1$ mg/kg. The concentration of Pb in the drinking water of the experimental animals was 620 mg/l≈620 mg/kg. The daily water consumption per animal was about 7 ml/day, and therefore, the daily Pb dose per animal could be approximately $B=4.34$ mg/day. Extrapolating over the experiment and taking into account the value of gastrointestinal resorption coefficient $\eta=15\%$ [34], it is calculated that, in group 3, the entire quantity of Pb absorbed by the gastrointestinal mucosa and entering into the blood during the experiment might be $A_{Group3}=58.6$ mg. Coefficient η ($0\leq\eta\leq1$) is a dimensionless coefficient indicating what fraction of ingested metal dose resorbs in the digestive tract. Converted to concentration in milligrams per kilogram, and taking into account that the mean mouse body weight during the experiment was about 30 g, we obtained $A_{Group3}=x\ (t_0)=$ 1953 mg/kg. The parameters were fitted by minimization of χ^2 by the use of an iterative Gauss-Newton procedure [35, 36]. Thus, the following values were found—for group 3, $a_1=0.022$ day^{-1}, $a_2=0.001$ day^{-1}, $a_3=0.099$ day^{-1}, and $a_4=0.004$ day^{-1}; for group 4, $A_{Group4}=x\ (t_0)=459.6$ mg/kg, $a_1=0.022$ day^{-1}, $a_2=0.002$ day^{-1}, $a_3=0.099$ day^{-1}, and $a_4=$ 0.004 day^{-1}. Based on the value A_{Group4}, absorption coefficient $\eta=3.53\%$ was calculated for group 4.

Discussion

Clinoptilolite Characteristics

In Bulgaria, clinoptilolites of high quality occur [37, 38]. Table 2 shows that the molar ratio Si/Al was 5.97 in natural clinoptilolite and 6.22 in modified KLS–10-MA based on this clinoptilolite. A rather minor ratio (6.02) has the purified natural clinoptilolite used for the treatment of diarrhea in the form of the drug "Enterex", approved by the Cuban Drug Control Agency in 1995 [39]. A value of 5.98 has the Greek natural clinoptilolite successfully used for treatment of solutions containing Pb^{2+}, Cu^{2+}, and Zn^{2+} [9].

The ratio SiO_2/Al_2O_3 in zeolites is a marker of great importance to determine their acid stability. A greater SiO_2/Al_2O_3 ratio correlates with higher acid stability of the zeolites [40]. Thus, the specific structure and high SiO_2/Al_2O_3 ratio in clinoptilolite makes it the preferable sorbent in different acid solutions, including gastric juice. Tao et al. investigating Na-clinoptilolite established that it maintained structure stability in solution with pH value of 1.2 [7], while Zhou and Zhu indicated a premature collapse of zeolites NaY and NaA in such strong acid solution [41].

The most important feature of zeolites, which determines their wide usefulness, is their cation exchangeability [42]. The aluminum ion is small enough to occupy the position in the center of the tetrahedron of four oxygen atoms, and the isomorphous replacement of Al^{3+} for Si^{4+} raises a negative charge in the lattice [21]. The negatively charged framework counter-balanced by positive cations (Na, K, and Ca), results in a strong electrostatic field on the internal surface. These cations can be exchanged to fine-tune the pore size or the adsorption characteristics. All clinoptilolite modifications are based on these principles. The pore size has an essential function in the Na^{+}, K^{+}, or Ca^{++} exchange with certain cations such as Pb^{2+}, Cd^{2+}, Zn^{2+}, etc. in solutions.

Regarding the selectivity of some metal and other cations towards the Bulgarian natural clinoptilolite, the following order has been established [38]:

$$Cs^{+} > K^{+} > NH_4{}^{+} > Ba^{2+} >> Sr^{2+} > Se^{2+} > Na^{+} > Ca^{2+} > Fe^{3+} > Pb^{2+} > Al^{3+} > Hg^{2+} > Mg^{2+} > Li^{+}$$

The above relations show that, practically, Pb occupies the first position among the metals encountered in industry and therefore, in the environment. For this reason, the main use of clinoptilolites for metal adsorption deals on the base of Pb.

The sorbent KLS–10-MA used in the present experiment is a Na-enriched sorbent. Tao et al. established that the structure variations of Na-clinoptilolite in the acidic solution for 24 h would have a minor influence [7]. The cation exchange capacity of KLS–10-MA was almost 1.4-fold higher compared with that of the natural clinoptilolite (Table 3). The exchangeable sodium cations in KLS–10-MA were 5.7-fold more, and the total exchangeable cations were 1.9-fold more than those in the natural matter. Results of several authors indicate that Na-enriched form of modified clinoptilolite has highest static ion exchange ability towards Pb^{2+}, Cd^{2+}, $NH_4{}^{+}$, etc. [7, 43–45]. Thus, the modification KLS–10-MA could be considered as a successful sorbent for detoxification purposes.

KLS–10-MA Significantly Reduces Pb Bioaccumulation in Carcass, Tissues, and Feces

As a feed additive, clinoptilolites have been used so far in poultry and livestock in order to positively influence feces consistency, reduce diarrhea, bind mycotoxins and aflatoxins, allow better performance of intestinal microflora [30], and minimize the negative effects of odor and other gaseous emissions such as NH_3 and H_2S [40]. Clinoptilolite with low toxicity and the high adsorption capacity toward Pb^{2+} ion in a strong acid solution has potential for application in life sciences [7]. The present work is the first exploration of the effect of clinoptilolite, used as a food supplement, in Pb-exposed animals.

It is known that the total body burden of Pb is divided into two pools, which have different rates of turnover. The largest and kinetically slowest pool is the skeleton, with a half life of more than 20 years, and a much more labile pool (about 20 days) is the soft tissue [1, 34]. Lead occurs in bone in much higher concentration than in other organs [34, 46] because it is a strongly bone-seeking element [47, 48]. The progressive accumulation of Pb in the bones is due to the inert character of bone tissue making the reverse resorption almost impossible. The obtained data confirm this state (Figs. 3 and 4). On day 90, $Pb_{Bone}/Pb_{Carcass}=5$ in group 3 and $Pb_{Bone}/Pb_{Carcass}=6.9$ in group 4.

The ratio Pb_{90}/Pb_{15} could be denoted as *bioaccumulation coefficient*. It is clear (Fig. 6) that the bioaccumulation coefficients in exposed and unsupplemented mice are much higher than in exposed and supplemented ones. The relations (5) and (6) exhibit the significant reduction of this coefficient, especially for the liver. It is once again an evidence for the significant drop of Pb bioaccumulation caused by the ion exchange capacity of the sorbent KLS–10-MA, hence for its effective detoxification function.

The ratios (7a) and (7b) show that kidney/liver Pb ratio was almost not influenced by the supplementation. Obviously, this fact is related to a strong relationship between liver and kidney Pb levels. In group 4, bone/liver Pb ratio was 2.1-fold higher and bone/kidney Pb ratio was 2.56-fold higher compared with group 3. This fact clearly indicates that the clinoptilolite sorbent exerts a significant detoxification effect in the soft tissues.

The Eqs. 8a, 8b, and 8c showed a highest reduction of Pb bioaccumulation, due to the supplement, in the kidney. This means that KLS–10-MA sharply decreases the Pb level in the blood. In fact, the sorbent acts in the gastrointestinal tract and significantly lowers the Pb-resorption by the mucosa. Thus, the present study confirms the assumptions of other authors that clinoptilolite sorbents can be reliable potential candidates for application in conditions of gastric juice [7].

The reduction of 88% of Pb content in the feces of the exposed and supplemented mice, compared with exposed and unsupplemented ones indicates that clinoptilolite supplementation can prevent, to some extent, the contamination of the environment.

The mathematical model of Pb kinetics is in good agreement with the experimental data for Pb concentration in bones (Fig. 4). Further resolution of this issue requires a more complex consideration of metal metabolism. The aim of the presented model was to indicate the main tendencies of Pb bioaccumulation in bones, taking into account only the basic factors determining this process.

The model shows that the rate constant of Pb excretion a_2 in group 4 is twofold higher than that in group 3: $a_{2\ \mathrm{Group}\ 4} = 2\ a_{2\ \mathrm{Group}\ 3}$. This result corresponds to the real situation because as a ballast matter, the clinoptilolite sorbent accelerates the intestine passage.

This model allowed determining the coefficient of Pb gastrointestinal absorption in the experimental animals from group 4, using the formula:

$$\eta = \frac{A_{Group_4} P}{1000BT} \tag{10}$$

where $A_{\mathrm{Group4}}=x(t_0)_{\mathrm{Group4}}$ is the concentration of lead absorbed in the digestive tract and entering the blood extrapolating over the experiment (for the mice of group 4), P is the mean mouse body weight during the experiment, B is the daily dose of Pb per animal, and T is the duration of the experiment. The value η=3.53% obtained in clinoptilolite supplemented mice (group 4) is 4.25-fold lower compared with η=15% in unsupplemented animals. A reduction of 76% occurred. In fact, this is a significant result: KLS–10-MA diminished the Pb absorption in gastrointestinal tract of mammals' organism more than four times.

Data of the investigation on chromosome aberrations, some blood parameters and body weight gain suggested that the used clinoptilolite sorbent was practically non-toxic [31]. A lack of toxic effects of clinoptilolite has been also demonstrated by other authors as well [15, 29, 40, 49].

Conclusion

Due to its structural stability under high temperatures and acidity, clinoptilolites are the most widely used natural zeolite in animal studies. The obtained results showed that the modified natural clinoptilolite sorbent KLS–10-MA, a Na-enriched alkali earth clinoptilolite, strongly decreases the absorption of lead in animals' digestive tract and thus limits Pb quantity entering the blood. The mice exposed to Pb and supplemented with KLS–10-MA exhibited a reduction in Pb levels in several samples of about 77–90%. The bioaccumulation coefficients Pb_{90}/Pb_{15} for carcass, liver, kidney, bones, and feces in supplemented animals were significantly lower compared with those in unsupplemented ones. These results indicate that the used clinoptilolite sorbent is quite effective in gastric juice medium and could be suitable for wide application in mammals under conditions of Pb poisoning. The mathematical model of Pb bioaccumulation in bones can predict the time course of Pb concentrations in conditions of chronic exposure to Pb with/without sorbent supplementation. The model also allows determining some kinetic parameters, especially; it was useful to calculate the coefficient of Pb gastrointestinal absorption in the case of sorbent-supplemented animals. This work gives ground to create a drug based on the clinoptilolite sorbent for supplementation of animals in regions that are industrially polluted with heavy metals, and particularly with Pb, in order to protect the animals' health and the quality of the environment.

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