Faecal Excretion Dynamic during Subacute Oral Exposure to Different Pb Species in *Rattus norvegicus*

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Abstract Faecal excretion is a basic means of detoxification upon ingestion of Pb-contaminated feed. In order to determine a time course of Pb elimination after oral exposure to two different forms of this heavy metal (lead acetate vs. phyto-bound Pb), a feeding study was carried out in experimental rats using the Pb phyto-hyperaccumulator *Pistia stratiotes* as a model diet. The effect of starvation on Pb excretion was further studied in rats that were fed plant material. Twelve Pb doses (7 μg Pb/1 g BW) were administered orally over a 5-week period. Faeces samples were collected 24 and 72 h post-exposure. Inductively coupled plasma optical emission spectrometry and electrothermal absorption spectrometry methods were used for determination of heavy metal concentrations. Up to 53 % of ingested Pb was rapidly eliminated from the exposed rats via faeces within 24 h after exposure. Faecal excretion in exposed rats differed significantly when compared to that of the control group. Fasting before exposure reduced Pb excretion by up to 50 %. Faecal excretions of both examined Pb forms exhibited almost identical patterns. Considerable differences were revealed concerning total excretion levels; lead acetate was excreted in amount greater extent than those of phytobound Pb. Results of our study suggest that Pb forms occurring in the *P. stratiotes* tissues are absorbed through the gastrointestinal tract to a greater extent than Pb from lead acetate. Therefore, higher portions of ingested Pb can be available for potential accumulation in tissues of exposed subjects.

Keywords Feeding study · Pb metabolism ·
Phytohyperaccumulator · Lead acetate · Excrement Pb level ·
Pistia stratiotes

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Introduction

Lead is the most abundant, persistent and widely distributed risk element in the environment, as confirmed by a United Nations Environment Programme [1] report stating that 18–22 million people worldwide are at risk from Pb poisoning. Pb-contaminated air, water, and soil may be incorporated into plant and animal tissues. Moreover, if Pb-polluted areas are used for agriculture (crop production or farm animals grazing), Pb enters biological tissues, and can be readily transferred to humans or animals through the food chain [2]. Therefore, heavy metals have been classified as major food contaminants for many years [3]. The basic principles of toxic metal (including Pb) intake from contaminated grasslands were described in a review by Wilkinson et al. [4] and a wide range of case studies confirmed elevated Pb tissue levels in livestock grazing in contaminated pastures [5, 6].

Most plant species (including crops) cannot survive on polluted sites due to the toxic effects of heavy metals; conversely, many plants are tolerant to this type

of contamination [7]. Some plant species even have plant accumulator properties. They can survive in highly polluted environments, and accumulate elevated concentrations of Pb in their tissues [8]. It is a worrying fact that some Pb–plant accumulators can be used as components of animal feed mixtures or as dietary supplements for humans [9]. For example, Ayoade et al. [10] recommended using aquatic lettuce (Pistia stratiotes L.) in pig fodder; however, numerous recent studies have confirmed Pb-accumulative properties of this plant [11–13]. High Pb levels in farm animal food and grain pose a significant risk because the consumption of Pb-containing products increases lead body burden and causes serious health problems.

The toxicokinetics, toxicodynamics, and physiologically principles of lead metabolism have been widely investigated [14, 15]. However, previous laboratory experiments were focused primarily on oral intake, alimentary tract absorption, metabolism, and excretion of separate Pb compound such as lead acetate [16], lead nitrate [17], lead sulphide, cysteine [18], triethyllead, and diethyllead [19]. Only a few feeding studies have dealt with the bioavailability and metabolism of different Pb species which occur in plant materials e.g. grass from natural pastures [20], forage [21], carrot [22], potato, and beetroot [23]. Therefore, available information concerning the course of these kinds of Pb compounds in the digestive tract can be considered insufficient.

Regardless of the exposure route, Pb is excreted primarily through the intestinal tract. Therefore, faeces (together with bile) are the basic means of detoxification [24, 25]. Hence, it is essential to obtain the relevant information about time course of phytobound Pb elimination from an animal or human body. The aim of our study was to determine differences in faecal Pb excretion dynamics during subacute oral exposure as affected by (1) different Pb form (inorganic Pb salt vs. lead hyperaccumulating plant P. stratiotes L.) and (2) starvation prior to exposure. Adult albino male rats were used as model organisms.

Materials and Methods

Chemicals Used in Experiment

Lead acetate, thrihydrate–$Pb(CH_3COO)_2 \cdot 3H_2O$ (p.a. grade, Lachema, Brno, Czech Republic)

Lead nitrate–$(Pb(NO_3)_2)$ (p.a. grade, Lach-Ner, Neratovice, Czech Republic)

Nitric acid–HNO_3 (p.a. grade, Lachema, Brno, Czech Republic)

ASTASOL–Standard lead solution (Analytika, Czech Republic)

Ammonium dihydrogen phosphate GR–$(NH_4)H_2PO_4$ (Suprapur grade, Merck, Germany)

BCR certified reference materials CRM 281–rye grass (IRMM, Geel, Belgium)

Maintenance of Experimental Animals

Twenty-seven male Wistar rats (Rattus norvegicus), in the age of 4 months (body weights are given in Table 2), were obtained from a commercial supplier (Institute of Physiology of the AS CR, Prague, Czech Republic). The rats were placed separately into plastic metabolic cages, and left to acclimatise for 7 days. The cages were kept at 22±2 °C in 12-h light/dark cycles, and the animals were fed commercial pellet food (St-1 by Velaz, Prague, Czech Republic) and were allowed to drink tap water at libitum. Additionally, 10 g of raw minced meat was administered twice a week in order to check if the animals will eat it readily. Thereafter, minced meat was used for Pb dose administration during the exposure period. For diet Pb details, see Table 1.

Experimental Design

All rats were randomly divided into four groups and treated according to Table 2. Lead was administered orally through two different treatments: metal salt–lead acetate–$Pb(CH_3COO)_2 \cdot 3H_2O$ (p.a., Lachema, Brno, Czech Republic) and the Pb hyperaccumulator plant P. stratiotes L. Plants were purchased from the Charles University Botany garden (Prague, Czech Republic) and cultivated in 60-L PE containers with Pb-enriched medium ($Pb(NO_3)_2$) (Lach-Ner, Neratovice, Czech Republic) under greenhouse conditions. Voucher specimens of Pistia plant, used in experiment, are deposited at the Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Science, Prague. Metal salt solutions were prepared by dissolving lead acetate (crystalline substance) in 1 ml of distilled water, vortexed for several seconds, and instilled into 10 g of raw minced meat. Plant dosages were prepared by mixing 10 g of raw minced meat and an appropriate amount of P. stratiotes L. dry mass (entire plants—comprising roots and leaves) milled to fine powder. The single dosage consisted of 7 μg of Pb per 1 g of rat body mass. Pb doses were administered at 3-day intervals (one dose every 72 h) continued for 5 weeks. For this

Table 1 Diet Pb levels (in milligrams per kilogram)

	Pb
Pellet food	1.87
Pistia stratiotes L.	41475
Minced meat	0.06
Drinking water	0.0005

Determined by inductively coupled plasma optical emission spectrometry and expressed as the mean of three measurements

Table 2 Experimental design

| Group code | Number of animals | Rat body weights[a] | | Dose (7 µg Pb/g BW) | Starvation before each exposure |
		Initial	Final		
O	6	467±51	476±35	0	No
LA	6	452±23	455±13	12× lead acetate	No
PH	9	464±33	465±34	12× plant hyperaccumulator	No
PHS	6	467±27	472±17	12× plant hyperaccumulator	Yes

[a]Mean±SD in a particular group

reason, rats were weighed prior to each exposure. A starvation effect was established by removing all available food from the feeders 12 h prior to each following exposure.

Sampling and Analytical Procedures

During the experiment, the rats were kept in individual metabolic cages for separate faeces collection. Faeces samples were collected 24 and 72 h after each Pb dosage into polyethylene containers and stored in the refrigerator until further procedures and analyses. To avoid Pb contamination, all sample containers used in the experiment were washed for 3 days in 5 % HNO_3 (p.a., Lachema, Brno, Czech Republic), and 1 day in distilled water. Faecal weight (in grams) was measured immediately after individual sampling. Faecal samples were dried for 12 h in a hot air oven at 108 °C to specify the weight of the dry mass, and to prepare the material for consequent mineralization. Afterwards, particular samples were pulverised and homogenised in a zirconium oxide grinding jar. For the determination of total Pb concentration in the faeces, experimental plants and diet, aliquots (1 g) of the dried and powdered samples were decomposed in 50 ml quartz–glass beakers at 500 °C for 16 h on a hot plate and in a muffle furnace with a stepwise increase of the ashing temperature [26]. Analytical blanks (total, 32; 5 % of all analysed samples) were prepared under the same conditions but without biological material. Blanks represent whole analytical process from the mineralization to Pb concentration measurement and were used to determine the analytical detection limit given as a triple standard deviation of blanks (listed in Table 3).

The total content of lead in the faecal digests was determined by inductively coupled plasma optical emission spectrometry (VARIAN VistaPro, Varian, Australia). The low concentrations of lead (e.g., in control

samples) were measured by electrothermal absorption spectrometry (ETAAS) using a Varian AA 280Z (Varian, Australia) with a graphite tube atomizer GTA 120 and a PSD 120 programmable sample dispenser. Standard lead solution ASTASOL (Analytika, CR), comprising of 100 mg Pb/l in 5 % nitric acid (v/v), with purity of Pb 99.999 %, was used in the preparation of a calibration curve for the measurement. The range of calibration curve for inductively coupled plasma optical emission spectrometry was 01.1–1 mg Pb/l. The evaluation of the concentrations for ETAAS was done using a standard addition method (bulk standard concentration, 60 µg/l), and ammonium dihydrogen phosphate GR (Merck) was used as matrix a modifier. The analytical procedure was validated using BCR-certified reference materials CRM 281–Rye grass (IRMM, Geel, Belgium). The measured values of CRM correspond to the range recommended for particular certified material. Due to the high number of samples, instrumental analyses were separated into four stages. The detection limits for each stage (mean ±3 SD of blanks) and the results of analytical quality assessment are given in Table 3. Pb concentrations determined below the detection limits were replaced by the value of detection limit in particular stage for further data processing. Multiplying faecal lead concentrations (in microgram of Pb per gram faecal dry weight) by the total faecal output (in gram dry weigh) determined the total faecal Pb output (in microgram).

Statistical Procedures

The amount of Pb in faeces was expressed as a ratio of oral dose to excreted Pb in order to attain individual results, which were comparable across the entire data set. Extreme values (up to three times interquartile range in each group),

Table 3 Detection limits (in microgram per liter) and results of CRM 281 (rye grass) analysis

	Pb value	Stage I	Stage II	Stage III	Stage IV
Detection limit (mean±3 SD of blanks)		14.25	3.45	10.23	4.26
CRM 281 rye grass (mgkg^{-1})					
Certified	2.38±0.11				
Found	2.48±0.13				

were excluded from further analyses. Variables were examined for normal distribution using the Kolmogorov–Smirnov test. Because the data were not normally distributed, nonparametric statistical procedures were used. The effect among groups was checked using Kruskal–Wallis test and pairwise multiple comparisons. The difference between results obtained 24 and 72 h after oral exposure was compared using Wilcoxon's signed-rank test. To find out whether faecal excretion dynamics differ among particular treatment methods, differences between the paired values (24 and 72 h Pb) were computed. The obtained differences were than compared using Kruskal–Wallis method with subsequent multiple comparison. Single categories are described using median, standard deviation, or bottom and top quartile. Analysis was performed using IBM SPSS Statistics, v. 19. Effects were considered significant if $p < 0.05$.

Results

A total of 648 faeces samples were collected during the experiment. Data concerning dietary lead intake and faecal Pb levels are given in Table 4. Pb intake from pellet food alone was 100 times lower than from an experimental oral dose of both lead acetate and plant hyperaccumulator.

Faecal Pb Levels

The highest faecal Pb concentrations were detected in both exposed groups (LA and PH) with normal feeding regimes (especially during the first 24 h after dosage). Faecal excretion was considerably reduced (by 50 %) in rats starved 12 h prior to Pb administration. It should be mentioned that results varied greatly within this study group.

Table 4 Pb dosages, food Pb intakes, and amount of Pb excreted in faeces (μg)

Group	Average Pb dose	Total Pb intake	Amount of Pb excreted in faeces		
			0–24 h	25–72 h	Total FE
O	30[a]	1,200[b]	3.91±4.09	3.65±3.82	7.20±6.37
LA	2,900±230	34,813±1,020	1,294±714	674±636	1,886±843
PH	3,000±260	35,951±2,820	1,157±490	431±404	1,572±546
PHS	2,930±220	35,120±1,900	580±472	112±208	676±533

Values expressed as the mean±standard deviation in the group

FE faecal excretion

[a] Calculated: amount of Pb in pellet feed×average day feed consumption per one rat

[b] Calculated: average feed consumption per 1 day×duration of exposure (40 days)

Faecal Excretion Dynamics

A total of 302 (collected 24 h after Pb dosage) and 305 (collected 72 h after Pb dosage) samples were the subject of statistical processing. Excretion dynamics were determined in cases where both values were obtained (296 pairs). Values are listed in Table 5.

At low Pb exposure (control group), faecal excretion ranged below 10 % of Pb intake in both sampling intervals (no significant differences were found between 24 and 72 h), and a total of 17 % of ingested Pb was eliminated via faeces. During the course of elevated (hundredfold) Pb exposure, the total excreted portion increased radically (two-, three-, and even fourfold to 28, 53, and 73 % in the PHS, PH, and LA groups, respectively). Moreover, regardless of the exposure variant, significant decreases were revealed between Pb portions excreted 24 (25.5–52.6 %) and 72 (1.6–16.1) h post-exposure; all exposed groups excreted the highest portion of the Pb dose in the first 24 h after oral intake (in LA group more than half of the dose). Regarding exposed groups, the food-restricted group eliminated significantly the lowest portion of the Pb dose in both sampling intervals, and faecal Pb excretion dropped to less than 2 % within 25–72 h post-dose administration. Generally, lead acetate was excreted to a greater extent than plant-bound Pb. Actual Pb excretion values for the LA and PH groups differed considerably. However, differences between lead acetate and plant hyperaccumulator group did not reach a statistical significance either in 24 or in 72 h faecal Pb ratios (complete overview illustrated in Table 6 and Fig. 1).

When comparing 24 and 72 h sampling intervals, the most significant decrease in Pb excretion was detected in group PHS (almost 16 times lower). In both exposed groups with normal feeding regimes, the 24/72 h decrease factor hovered around 3–4. The control group showed approximately the same values in both sampling intervals due to a stable Pb intake via food pellets. Therefore, the dynamics of Pb faecal excretion differed significantly among control and all exposed groups. Even though different LA and PH Pb ratios were detected, faecal excretion of studied Pb forms (lead acetate, plant material) exhibited similar patterns (Fig. 2).

Discussion

Both wild and farm animals are often exposed to elevated Pb intake through the forage and the adverse effects of long-term exposure to Pb-contaminated feed were reported by many authors [5, 27]. Wilkinson et al. [4] detected that grass meal from Pb polluted areas may contain more than 40 mg Pb/kg. These Pb concentrations exceed the maximum permissible limits for animal feed in the EU (10 mg Pb/kg) as well as in the USA (30 mg Pb/kg) [28, 29]. Nevertheless, the

Table 5 Pb excretion (% of Pb dose excreted in faeces)

Group	% of Pb dose excreted in faeces					Decrease factor[a] (*p* value WSR)	Total faecal excretion (%)
	n	0–24 h	*n*	25–72 h			
O	69	9.59[LA, PH, PHS] (3.99–17.0)	67	7.62[LA, PHS] (4.30–14.1)		1.3[NS]	17.3[LA, PH]
LA	68	52.6[O, PHS] (23.1–64.0)	71	16.1[O, PHS] (8.51–30.4)		3.3[<0.0001]	73.0[O, PH, PHS]
PH	105	38.8[O, PHS] (28.6–48.3)	105	10.4[PHS] (3.79–19.8)		3.7[<0.0001]	53.2[O, LA, PHS]
PHS	60	25.5[O, LA] (8.16–46.2)	62	1.60[O, LA, PH] (0.82–3.65)		15.9[<0.0001]	28.2[LA, PH]

Values expressed as median (top–bottom quartile) in the group

n quantity of variables in particular group, *WSR* Wilcoxon's signed-rank test, *NS* not significant

[a] Calculated 24/72 h faecal Pb

O, LA, PH, PHS significantly different ($p<0.05$) compared to the indexed group (Kruskal–Wallis)

course of phytobound Pb in the food chain has yet to be described in detail. Therefore, the result of our study should contribute to clarify the excretion dynamic of Pb from plant feed with elevated Pb levels.

Total Faecal Pb Excretion

The majority (approximately 90 %) of Pb oral intake is usually excreted via faeces in rats [30]. In the present study, lower total faecal excretion ratios were detected. Lead acetate excretion level (73 %) was similar to that of plant-bound Cd (75 %) in rats [31] or in sheep [32] fed Cd-contaminated feed; determined within 4 days post-exposure. However, values of *Pistia*-bound Pb excretion (53 %) from our experiment were lower when compared to those from all studies mentioned above. This difference was most likely due to the shorter sampling period used in our experiment. The variations in Cd and Pb intestinal absorption levels may be another explanation for this divergence. However, there is a lack of data concerning faecal excretion of Pb from the plant hyperacummulator for direct comparison with our

results. Anyway, such low levels of plant-bound Pb excretion indicate that a greater amount of this element would probably be retained in the body of the exposed subjects.

Effect of a Pb Dose and a Starvation on Faecal Pb Excretion

The present study demonstrated the differences between faecal Pb excretions in the course of low and elevated oral exposure to various Pb forms. All groups with a high Pb intake eliminated two to four times more ingested Pb than that of the control group. This can be attributed to the fact that lead absorption from the gastrointestinal tract seems to

Table 6 Kruskal–Wallis tests detailed results with subsequent pair-wise multiple comparison

Group	Faecal excretion			
Comparison	0–24 h	25–72 h	Dynamic[a]	Total[b]
0–LA	<0.001	0.025	<0.001	<0.001
0–PH	<0.001	NS	<0.001	<0.001
0–PHS	<0.001	<0.001	<0.001	NS
LA–PH	NS	NS	NS	0.002
LA–PHS	0.002	<0.001	NS	<0.001
PH–PHS	0.026	<0.001	NS	<0.001

NS not significant

[a] Calculated: % 24 h/% 72 h, differences compared among particular groups
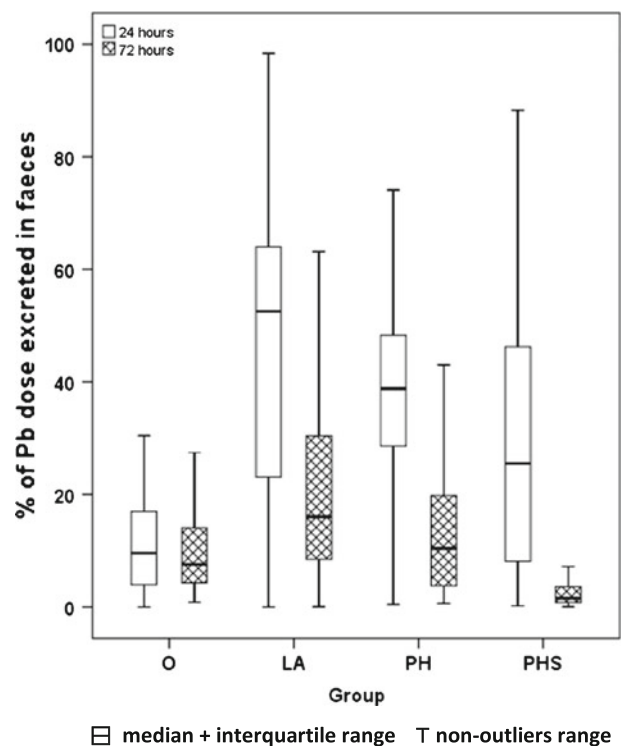
[b] Calculated: % 24 h+% 72 h

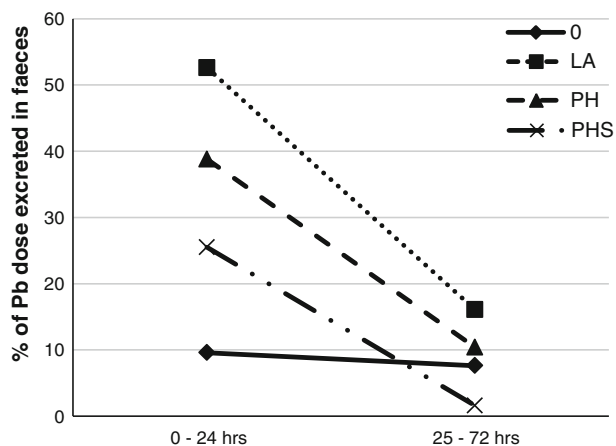**Fig. 1** Portion of Pb dose excreted in faeces

Fig. 2 Pb excretion dynamic

be a capacity limited process, and the percentage of ingested lead absorbed may decrease as lead intake increases [15, 17, 33]. This phenomenon can be described as a means of self-preservation, because even at high Pb concentrations in food, its absorption from the gut into the body is limited and most of contaminant is excreted. The dose at which absorption of Pb becomes appreciably limited is yet to be known. Therefore, it is always necessary to note the exposure level or dose at which excretion or absorption is observed.

Rabinowitz et al. [18] found that starvation during oral exposure to lead decreases Pb excretion by 30 %. A very similar pattern can be observed in our experiment, where forced starvation reduced Pb faecal excretion from 53 to 28 %. Decreased Pb excretion is most likely due to a higher intestinal absorption of this metal during fasting or nutritional disbalance described by Heard [34] and Blake [35]. Our results suggest that, in this respect, Pb incorporated in plant tissues behaves like other lead compounds used in previous experiments (Pb acetate, nitrate, etc.) as its intestinal absorption can increase during starvation.

Pb Excretion Dynamics

According to previous studies dealing with medium-term excretion dynamics after oral Pb (lead nitrate, lead cysteine) or Cd intake [18, 31, 32], faecal excretions of ingested heavy metals are greatest within the first 5 days after exposure (84 %), and then decrease rapidly to approximately 5 % in the following days. The dynamics of Pb faecal excretion of both Pb forms investigated in our study were considered as consistent with those of the works listed above.

Contrary to Rabinowitz et al. [18], we focused our attention on short-term excretion dynamic. Through the use of 24- and 72-h sampling periods, we were able to reveal a different excretion patterns in particular treated groups and to formulate decreased factor for faecal Pb, which was not been determined in previous scientific studies. During

elevated Pb exposure, the highest portions of ingested Pb were eliminated via faeces in 24 h post-administration. Thereafter, the excreted portion significantly decreased. The most radical difference was detected in the fasted group (degrease factor between 24 and 72 h, faecal Pb=16); 72 h post-administration this group excreted even five times lower portion of ingested Pb when compared to that of control group. This is most likely the result of elevated lead intestinal absorption during fasting (discussed above). Excretion dynamic of lead acetate and *Pistia*-bound Pb showed almost identical pattern. However, 72 h post-exposure Pb portion excreted in PH group was very close to that of control group. This finding suggests that while excretion of lead acetate is quite rapid even in 3 days after the intake, plant-bound Pb is at this time period excreted on a similarly low level as in the course of low exposure.

Effect of Pb Form on Faecal Pb Excretion

Previous studies determined the Pb excretion dynamics particularly after intravenous administration [24, 25]. Therefore, direct comparison with these works could be misleading as the toxicokinetic of Pb in the body is strongly dependent on the route of exposure [36]. Contrarily, recent feeding studies dealing with Pb-contaminated feed intake were focused primarily on tissue absorption and did not include data concerning Pb elimination from the body. Therefore, an important point obtained in our study is the definition of faecal Pb excretion after ingestion of phyto-bound Pb. Even though no statistically significant differences in faecal excretion dynamics of individual Pb forms were detected, considerable differences were revealed concerning the total excretion levels of Pb bound in plant material compared to those of lead acetate. Lead acetate was eliminated from the body of experimental rats more rapidly than Pb from a plant hyperaccumulator. These findings suggest that Pb forms occurring in the *P. stratiotes* tissues are absorbed through the gastrointestinal tract to a greater extent than lead acetate, and higher proportions of ingested Pb can accumulate in animal tissue. The reason behind these different excretion/absorption levels can be found in different chemical characteristic of Pb compounds occurring in plant hyperaccumulators.

In phytohyperaccumulators, heavy metals detoxification does not rely on high molecular mass ligands, such as phytochelatins in nonhyperaccumulator plants [37, 38]. Hyperaccumulator tissues possess large quantities of small organic molecules that can operate as metal-binding ligands and bivalent cations (Pb^{2+}) bind to free amino acids, such as histidine and nicotinamine [39]. Yet only few research studies dealing with the identification of Pb compounds in other plant hyperaccumulators can be found. Pb accumulates as lead acetate, sulphate, or sulphide in tissues of the Pb

hyperaccumulator *Sesbania drummondii* (Fabaceae) [40] or as cerussite (lead carbonate) in *Phaseolus vulgaris* (Fabaceae) [41] grown in a Pb-enriched medium. Concerning *P. stratiotes*, Odjegba and Fasidi [12] localised all heavy metals and risk elements (including Pb) accumulated mainly in the root system. However, specification of Pb compounds in this plant remains unclear and additional research focused on this topic should be carried out. With respect to previous studies, we can only assume that Pb forms occurring in *P. stratiotes* are similar to those of other plant hyperaccumulators. This assumption is supported by the fact that amino acids such as tyrosine, arginine, or cysteine increase intestinal Pb absorption [42]. Also, absorption of Pb carbonate is higher when compared to that of lead acetate [43]. Therefore, Pb bound to sulphur or carbon compounds in plant hyperaccumulators can most likely pass easily through the intestinal epithelium and can be available for accumulation in animal tissues. Higher faecal elimination of inorganic form of heavy metal in comparison of that of plant-bound one was described by Phillips at al. [32] as well for Cd. Although, results of present study coincide with those findings, Rabinowitz et al. [18] did not observed significant results between ingestion of lead nitrate and lead cysteine, confirming the need for further research concerning bioavailability of risk elements bound in plant tissues.

Conclusions

In this feeding study, we found that the course of phyto-bound Pb (hyperaccumulator *P. stratiotes*) inside the gastrointestinal tract varied from that of standard heavy metal salt (lead acetate). Although faecal excretions (the main route of detoxification) of different Pb forms exhibit almost identical pattern, considerable differences were revealed concerning the total excretion levels of both investigated compounds. Despite expectations, lead acetate was eliminated from the body of experimental rats more rapidly than Pb from a plant hyperaccumulator. These findings suggest that Pb forms occurring in the *P. stratiotes* tissues are absorbed through the gastrointestinal tract to a greater extent than Pb from lead acetate, and higher portions of ingested Pb can be accumulated in tissue of exposed subjects. However, information concerning the particular chemical speciation of Pb incorporated in *P. stratiotes* tissues is lacking, and additional research focusing on identification of these compounds should be carried out. Also, bioavailability and bioaccumulation levels of Pb from hyperaccumulator plants are yet to be verified in subsequent studies concerning blood, serum, or tissues Pb level. Results of our experiment highlight the need for more comprehensive research on the course of phyto-bound Pb inside the gastrointestinal tract, as these Pb accumulator plants can be part of animal or human diet.

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