ADVANCING CADMIUM QUANTIFICATION IN CELLULAR COMPARTMENTS: A RADIOANALYTICAL APPROACH

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INTRODUCTION

Distribution and allocation of metals within different cellular compartments significantly impact plant responses to toxic elements like cadmium. While techniques like atomic absorption spectrometry are valuable for subcellular cadmium detection, they require substantial biomaterial quantities, making them less feasible for early developmental stages where sample sizes are limited. In response to this challenge, we have developed a radioanalytical method that allows for precise cadmium quantification in individual cellular compartments using much smaller tissue samples. By focusing on early developmental stage wheat, we analyzed the subcellular accumulation of cadmium using gamma spectrometry.

MATERIALS AND METHODS

Wheat plants were subjected to exposure from a cadmium solution (50 $mg.L^{-1}$ Cd^{2+}, enriched with $^{109}Cd^{2+}$). Following a cultivation period of 10 days, plant tissues were harvested, homogenized utilizing a laboratory mill in the presence of liquid nitrogen, and extracted within an inert solution. The resultant homogenate and subsequently, the supernatant, were further subjected to centrifugation following a specific protocol to yield distinct fractions. The initial sediment corresponded to the cell wall fraction (F1), the subsequent sediment (F2) represented the fraction containing cellular organelles, and the resulting supernatant contained the soluble (cytosolic) or vacuolar cell fraction (F3). The ultimate concentration of cadmium in both root and aboveground tissues was determined through scintillation gamma spectrometry.

AIM

The aim of this study was to optimize a method for subcellular localization and quantification of cadmium in wheat roots to reveal allocation and tolerance strategies among a set of wheat cultivars.

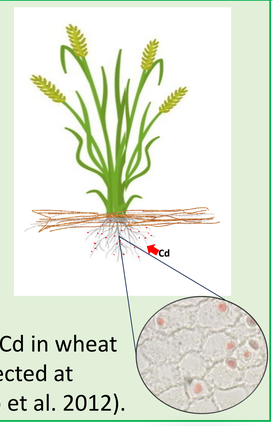

Figure 1 Accumulation of Cd in wheat roots – in the section detected at subcellular level (Konotop et al. 2012).

RESULTS

After conducting tissue centrifugation to separate fractions in the wheat model, we investigated the subcellular allocation of cadmium within the root tissues using gamma spectrometry. In different wheat varieties, we observed variation in the subcellular localization of metal, which differed also between roots and shoots. The findings indicated a robust association with specific physiological and biochemical markers that reflect wheat's responsiveness or tolerance to the harmful effects of Cd exposure. Allocation in cell wall coincides with higher Cd tolerance.

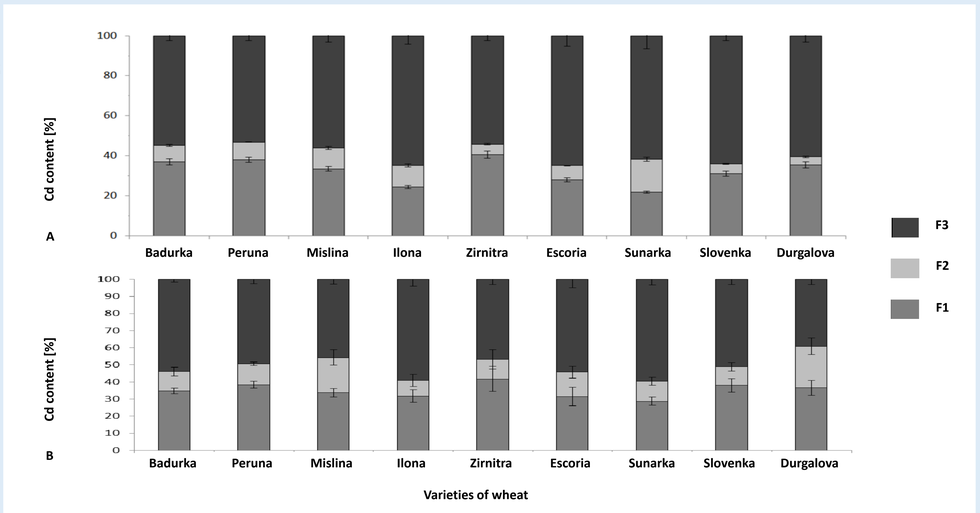

Figure 2 illustrates a visual depiction of the cadmium (Cd) accumulation ratio within specific cell fractions of both root tissues (A) and aboveground portions (B). These fractions include cell wall content represented by grey bars (F1), organelles depicted by pale bars (F2), and cytosol indicated by black bars (F3). The data presented here signify the average values along with the standard error (SE) based on a sample size of three (n = 3).

CONCLUSIONS

Tissue centrifugation allows for the separation of cell fractions, in which Cd content can be quantified using gamma spectrometry. The method requires little amount of plant material. The findings indicated a robust association with specific physiological and biochemical markers that reflect wheat's responsiveness or resistance to the harmful effects of cadmium exposure. These findings provide valuable insights into the sensitivity and tolerance of wheat to cadmium toxicity. Such knowledge is crucial for developing strategies to mitigate the adverse effects of metal toxicity, particularly in crops intended for organic farming. Ultimately, our approach opens new avenues for investigating metal accumulation dynamics in plants, facilitating the development of resilient and environmentally sustainable agricultural practices.

ACKNOWLEDGEMENTS

The work is supported by the projects APVV-21-0504 and KEGA 022UCM-4/2021.

EFFECT OF SUPERABSORBENT POLYMERS APPLICATION ON CROP GROWTH AND UPTAKE OF MICROELEMENTS

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INTRODUCTION

Drought represents one of the most natural disasters affecting the economic, social and environmental status and development of every country. It is currently being significantly exacerbated by ongoing climate change. Superabsorbent polymers (SAPs) represent a potential tool to alleviate drought stress in agriculture due to their ability to form a hydrophilic three-dimensional network that exhibits excellent water absorption and retention characteristics.

MATERIALS AND METHODS

Seeds of barley (*Hordeum vulgare* L.) variety BOJOS, maize (*Zea mays* L.) variety ALOMBO, rapeseed (*Brassica napus* L.) variety CORZAR or wheat (*Triticum aestivum* L.) variety LUANA untreated or treated with SAP in the form of thin films and/or fungicide (Agro Aquaholder Seed+, PeWaS Ltd.) were planted and grown in two experimental plots located in the Slovak Republic (Borovce; N 48°34'51.9", E 17°43'59.6") and the Czech Republic (Žabčice; N 49° 1.37702′, E 16° 37.07360) in 2021 and 2022. After approx. 6 weeks, plants were harvested, divided into aerial parts and roots, dried, homogenized and analysed gravimetrically or by ICP-MS (Aurora M90 Bruker, USA) for microelements Co, Cu, or Zn uptake. Soils from the above mentioned experimental plots were also sampled to determine their basic chemical parameters: TOC (TOC-V CPN Shimadzu, Japan), content of Ca, Fe, K, Mg, Mn, and P (ICP-AES Agilent 5100, USA), content of Co, Cu or Zn (Aurora M90 Bruker, USA), and S_{total} (Elemental determinator LECO 628S, USA). The statistical significance of the obtained results in terms of evaluating the differences between seed treatments in terms of crop growth parameters and microelements uptake was analyzed based on Duncan's multiple range test using STATGRAPHICS Centurion XV ver. 15.1.02.

AIM

The aim of this work was to investigate the potential role of SAPs applied on the seeds of barley, maize, rapeseed or wheat in the form of thin films (Agro Aquaholder Seed+, PeWaS Ltd.) in mitigating the effects of drought, whereby the effect of this treatment was evaluated on the basis of crop growth parameters and microelements uptake. Crops were grown in two experimental plots in the Slovak Republic (Borovce) and the Czech Republic (Žabčice) in 2021 and 2022.

RESULTS

Both localities of experimental plots were similar in terms of soil chemical parameters, such as TOC, content of K, P, Co, Cu, and Zn (Borovce – 1.89 %, 1.90 %, 0.090 %, 11.5 ppm, 23.8 ppm, and 76.3 ppm; Žabčice – 1.62 %, 2.21 %, 0.105 %, 13.8 ppm, 29.1 ppm, and 96.9 ppm). The years evaluated differed in the level of drought, within the both localities reduced soil moisture or incipient drought in 2021 was described, but in 2022 moderate to severe drought was identified (data from https://www.intersucho.sk/). In 2021, a statistically (at $p < 0.05$) significant positive effect of SAPs without or with fungicide application on crop growth was only observed for the Borovce locality and crops of barley and maize. In the following year 2022, when spring was characterised by a severe drought, a statistically positive effect on the crop growth was observed for seeds of maize coating with SAPs at both localities (Fig. 1). For all crops evaluated, the uptake of microelements decreased in the order: Zn > Cu > Co. The highest Zn uptake of 71.0 ppm was observed for plants of wheat and decreased in the order: wheat > barley > maize > rapeseed. In the case of Cu and Co, the highest uptake values were 20.4 ppm and 4.09 ppm, respectively, determined at maize. The values of transfer factors (*TF*s) were in the range of 0.02 – 0.34 for Co, 0.32 – 0.75 for Cu, and 0.43 – 0.90 for Zn. In terms of SAPs application, there were no statistically significant differences in the uptake of microelements and their translocation to aerial parts of plants.

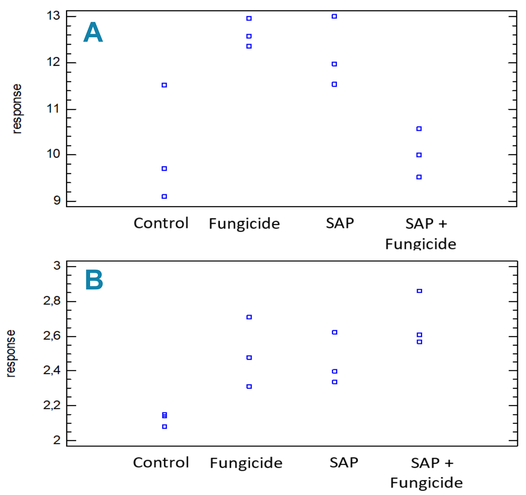

Fig. 1: Comparison of the growt of maize plants planted as untreated or treated seeds with SAPs and/or fungicide and cultivated in experimental plots in Borovce (**A**) and Žabčice (**B**) during 6 weeks in 2022. Results were compared according to Duncan´s multiple range test (at the $p < 0.05$).

CONCLUSIONS

The obtained results showed that SAP applied in the form of thin films to the seeds of the studied plants - maize, barley, wheat and rapeseed is able to effectively suppress the drought stress and thus soil moisture deficiency in the case of the occurrence of moderate to severe drought in the first stages of growth of the above mentioned crops. It was also shown that the application of SAP to seeds did not affect the uptake of important microelements (Zn, Cu and Co) nor their availability in the soil as evaluated by calculated *TF* values. Differences in the uptake of microelements were only observed at the crop level. Determination of activity of enzymes L-ascorbate peroxidase and catalase responsible for metabolizing stress-provoked reactive oxygen species (ROS; such as H_2O_2) demonstrated that the use of SAP significantly alleviated drought stress in plants (data not shown).

ACKNOWLEDGEMENTS

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ANS 2023

58. The impact of eight-week pre-interaction of poly-3-hydroxybutyrate with soil on its biochemical properties during eight-week plant growth under different soil-sand ratios

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INTRODUCTION

Poly-3-hydroxybutyrate (P3HB) is a bacterial biopolymer used for production of plastics, environmentally friendly alternative to traditional, long-persisting plastics, due to feasibility of its waste disposal via biodegradation. In contrast to previous expectations, it is not as much uninfluential: its plastic particles may promote undesirable changes in the proportion of soil nutrients and subsequently decrease plant primary production due to negative influencing of soil food webs. This study examined the effects of P3HB addition and dilution of soil with sand on soil biological properties those related to soil microbial composition, enzymes and soil respiration. The effects of P3HB addition on plant biomass were also compared.

MATERIALS AND METHODS

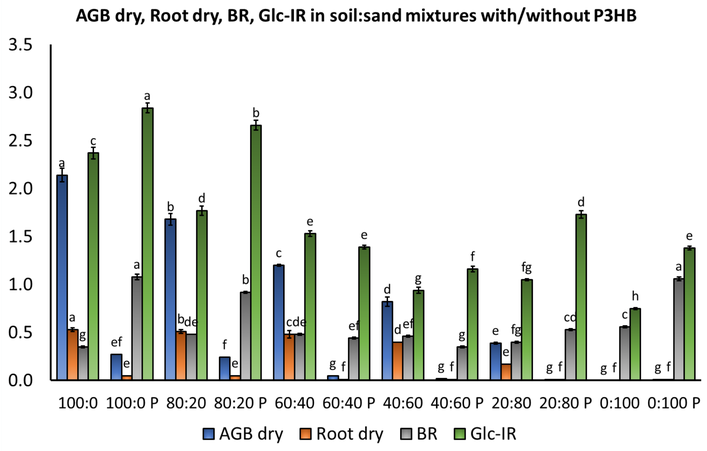
The study was based on the pot experiment with using Haplic Luvisol, a silty clay loam soil. The sieved soil was mixed with the with a fine quartz sand (0.1–1.0 mm; ≥95% SiO_2) in the following weight ratios: (I) 100% soil; (II) 80% soil + 20% sand; (III) 60% soil + 40% sand; (IV) 40% soil + 60% sand; (V) 20% soil + 80% sand; (VI) 100% sand. Each treatment was either (A) with 1 wt% P3HB or (B) without any amendment (control). P3HB pellets (< 63 μm) = ENMAT Y3000P were from TianAn Biologic Materials Co., Ltd. After 8-week pre-incubation of all (2×6 variants) pots, each one was seeded with sprouted lettuce (*Lactuca sativa* L.). Cultivation at full-spectrum LED lighting (20 klx)/12 h, temperature 18/22 °C (night/day) and relative humidity 70% was done for 8 weeks.

AIM

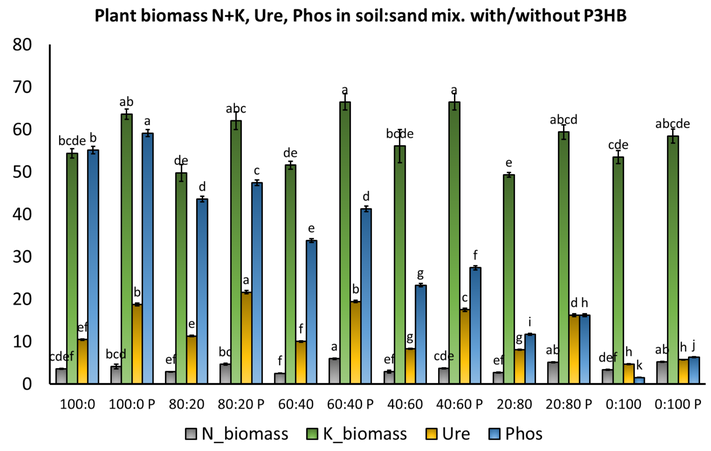
The study aims to answer the following questions:

a) How and to which extent the soil fertility and microbial communities are affected by the P3HB amendment?
b) How the soil „dilution“ influence on soil fertility and microbial activity proportionally?
c) How is the microbial activity affected by P3HB amendment after pre-incubation in absence of the lettuce plant?

RESULTS



Dry aboveground plant biomass (AGB dry, g/plant), dry root plant biomass (Root dry, g/plant), basal respiration (BR, μg CO_2/g dry soil weight d.w./h), D-glucose induced respiration (Glc-IR, μg CO_2/g d.w./h). Mean values (n = 3) ± standard error of mean (SEM) = error bars; letters indicate differences between variants calculated by Tukey‘s HSD posthoc test at a statistical level of significance p ≤ 0.05.



N content in plant biomass (N_biomass, mg/kg d.w.), K content in plant biomass (K_biomass, mg/kg d.w.), soil phosphatase (Phos, nmol PNP/g d.w./min.), soil urease (nmol NH_3/g d.w./min.). Mean values (n = 3) ± standard error of mean (SEM) = error bars; letters indicate differences between variants calculated by Tukey‘s HSD posthoc test at a statistical level of significance p ≤ 0.05.

CONCLUSIONS

P3HB addition significantly retarded the plant growth as indicated by reduced dry plant above and below ground biomass (in average by -79% and -91% compared to unamended soil). The PH3B addition at various sand:soil ratios enhanced N, K content in plant biomass (in average +55% and +5%) as compared to the content in sole soil. P3HB addition stimulated N and P acquiring enzymes urease and phosphatase (in average by +83% and +69%) compared to unamended soil. The soil basal and substrate-induced respirations were higher under P3HB addition and varied depending on sand:soil ratio. While soil shows ability to break down bioplastics, this occurs at the expense of nutrient availability to plants and with an adverse effect on their growth.

ACKNOWLEDGEMENTS

This research was supported by the projects FCH-S-23-8297, LTI20007 of Ministry of Education, Youth and Sports of the Czech Republic, and by the Ministry of Agriculture of the Czech Republic within the institutional support MZE RO1223 (Agricultural Research, Ltd. Troubsko) and MZE-RO1723 (Agrovyzkum Rapotin, Ltd.) in the frame of the Long Term Conception of the Development of the Research Organization.

ANS 2023 PROPERTIES AND BENEFITS OF SEWAGE SLUDGE-DERIVED BIOCHARS

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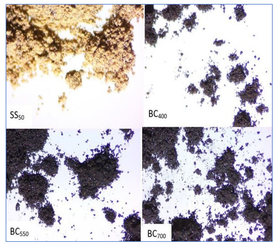
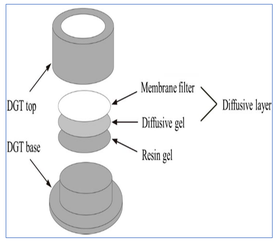
INTRODUCTION

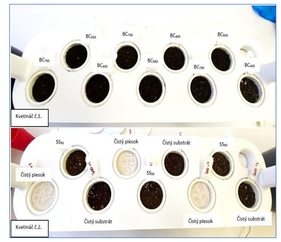
Pyrolysis represents promising thermochemical method for the processing of complicated waste such as sewage sludge. The thermochemical conversion reduces the total volume of sludge produced by WWTPs. Moreover, pyrolysis transforms sludge into valuable product in compliance with the objectives of the circular economy. However, despite the significant benefits of sludge-based biochar (high content of organic C, Zn, P), its use as a fertilizer has several limitations, mainly due to the relatively high heavy metals content in its structure. In general, heavy metal mobility, bioavailability or toxicity are determined by their chemical speciation. The pyrolysis temperature at which the materials were prepared is considered to be a key factor influencing the speciation of heavy metals in pyrolysis products and their bioavailability to plants. Therefore, the aim of the work was the physicochemical characterization of sludge-derived pyrolysis materials obtained by a slow pyrolysis process at varying temperature (400 - 700 °C) from distillery sewage sludge in order to assess their possibility of use as soil additives with a focus on the bioavailability of Zn.

MATERIALS AND METHODS

The anaerobic sludge coming from the WWTP of a bioethanol production plant (Enviral a.s., Leopoldov, Slovakia) was obtained from the IC reactor, filtered, washed and dried to constant weight at 50 °C (SS50). The treatment sludge was subjected to slow pyrolysis process at three different temperatures i.e. 400 (BC400), 550 (BC550) and 700 °C (BC700). The pyrolysis process was carried out in a muffle furnace (Nabertherm L15/11/B410) with a maximum capacity of 15 l. To ensure a strictly anoxic atmosphere during the pyrolysis process, N_2 gas was used with a flow rate of 1 l/min of the reactor exchange volume. The heating rate was 10 °C/min, while the residence time of the biomass in the reactor was 1 h.Obtained material was crushed, sieved to yield a fraction of 0.05 - 1 mm using standard sieves and characterized by determinatin of pH, EC, $CaCO_3$, CEC, ash content and RFA analysis for determination of total As, Cd, Cr, Cu, Ni, Zn, Fe and Hg concetrations. Mobile and bioavailable forms of Zn were characterized by DGT, DV and DTPA simultanous and BCR-sequential extraction protocols. Zn bioavailability was studied in short term pot-experiments with spinach (*Spinacia oleracea* L.*)* as a model biological object and smart pots. Obtained data were evaluated by statistical analysis ANOVA (Statistica, StatSoft-USA).







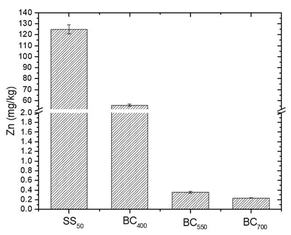
RESULTS

Physico.-chemical characterization

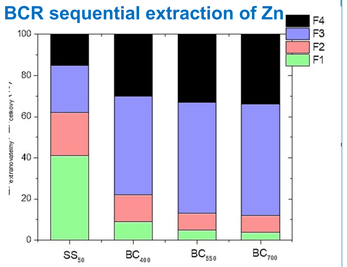
	SS$_{50}$	BC$_{400}$	BC$_{550}$	BC$_{700}$
pH$_{H2O}$	6,59 ± 0,20	5,11 ± 0,28	7,84 ± 0,28	12,29 ± 0,04
pH$_{KCl}$	6,25	5,31 ± 0,11	8,09 ± 0,09	10,39 ± 0,06
EC (mS/cm)	6,16 ± 0,23	3,55 ± 0,17	2,53 ± 0,11	1,83 ± 0,02
C$_{org.}$ (%)	**38,31**	**50,34**	**51,00**	**53,43**
C$_{total}$ (%)	39,81	51,38	52,27	54,92
C$_{anorg.}$ (%)	1,50	1,04 ± 0,07	1,27 ± 0,04	1,49 ± 0,01
H (%)	6,39	3,40	1,46	0,77
N (%)	9,94	9,89	9,48	5,28
ash (%)	14,00	30,35	38,26	46,38
VOC (%)	74,71	36,06	21,48	9,29
Zn$_{DGT}$ (mg/l)	3,308	4,473	0,819	0,8449

	SS50	BC400	BC550	BC700
As (mg/kg)	4	4	5	6
Cd (mg/kg)	< 1	< 1	< 1	< 1
Cr (mg/kg)	23	26	45	198
Cu (mg/kg)	153	124	238	295
Ni (mg/kg)	92	171	195	312
Pb (mg/kg)	6	7	7	9
Zn (mg/kg)	**717**	**1293**	**1626**	**1869**
Fe (mg/kg)	51700	75800	98000	116000
Hg (mg/kg)	< 2	< 2	< 2	< 2

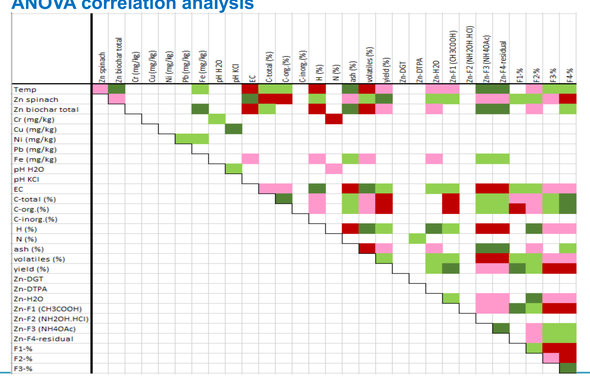
DV -extractable Zn



BCR sequential extraction of Zn



ANOVA correlation analysis



CONCLUSIONS

Physicochemical characterization of distillery sludge and pyrolysis products showed the active and potential pH values of the samples increased with increasing pyrolysis temperature. We observed a decreasing trend in the EC values of the samples and their volatile matter, H, N contents in the order SS$_{50}$ > BC$_{400}$ > BC$_{550}$ > BC$_{700}$. The ash, C$_{total}$ and C$_{org.}$ contents increased with increasing pyrolysis temperature at which the samples were prepared. Using the RFA method, we found that the concentrations of Zn as well as Cr, Cu, Fe and Ni in the samples also increased with increasing pyrolysis temperature. The most abundant metal was Fe, whose concentration in sample BC$_{700}$ reached up to 116000 mg/kg explained by the addition of coagulants and flocculants. The results of the BCR sequential extraction protocol showed that even though there was multiplication of total Zn in the samples with increasing pyrolysis temperature, the bioavailable fractions of Zn (F1, F2) were converted to more stable fractions (F3, F4) with low mobility and bioavailability. The results of the pot experiment confirmed this argument, as Zn accumulation by spinach plants decreased with increasing pyrolysis temperature at which the samples were obtained. Statistical confirmed the key effect of pyrolysis temperature on chemical speciation of Zn in the solid pyrolysis products.

ACKNOWLEDGEMENTS

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AGENTÚRA NA PODPORU VÝSKUMU A VÝVOJA

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SYNTHESIS OF TRICYCLIC 5:5:6 HETEROCYCLES WITH THREE HETEROATOMS

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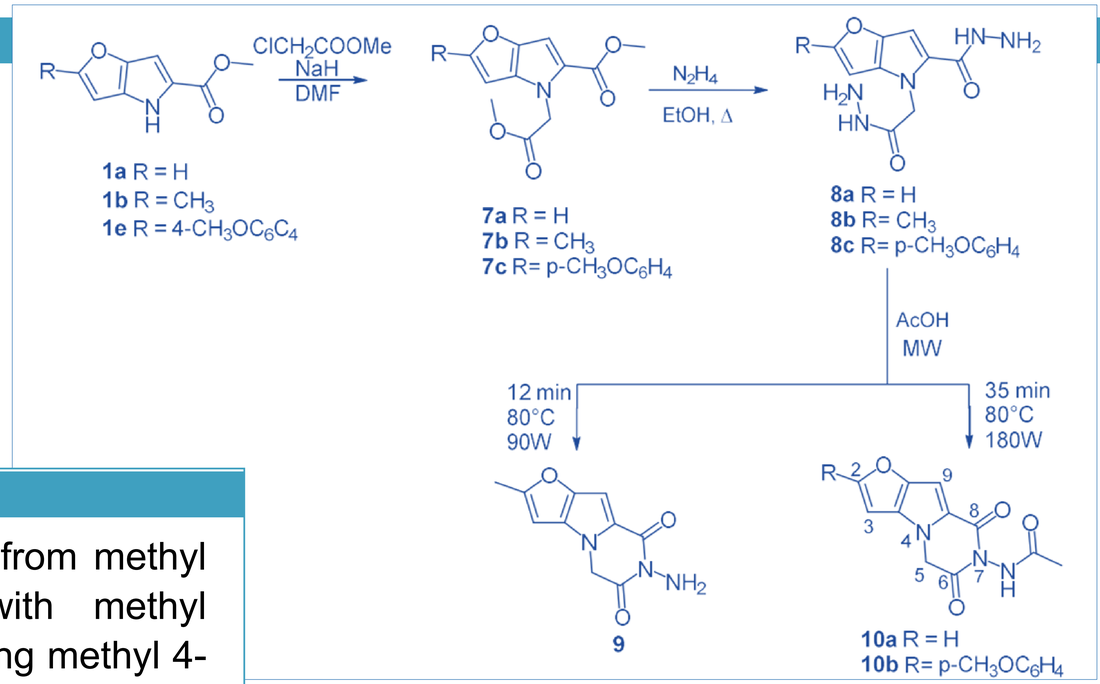
INTRODUCTION

Furo[3,2-*b*]pyrroles are isosteres of the indole ring system in which the benzene ring is replaced by the furan ring. Efficient synthetic routes to these heterocycles are of great interest as the furo[3,2-*b*]pyrrole core has been found in compounds with diverse biological activities or they are used as the fluorescent dyes.



1a R = H
1b R = CH_3
1e R = 4-$CH_3OC_6C_4$

7a R = H
7b R = CH_3
7c R= p-$CH_3OC_6H_4$

8a R = H
8b R= CH_3
8c R= p-$CH_3OC_6H_4$

9

10a R = H
10b R= p-$CH_3OC_6H_4$

RESULTS

Furo[2',3':4,5]pyrrolo[1,2-*a*]pyrazines **9** and **10** were synthesized from methyl 4*H*-furo[3,2-*b*]pyrrole-5-carboxylates **1** *via* their alkylation with methyl chloroacetate in DMF/NaH at room temperature overnight, providing methyl 4-(2-methoxy-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylates **7** in 61-67% yields. Subsequent hydrazinolysis led to the bis-hydrazides **8** in 65-70% yields. Final cyclisation of bis-hydrazides **8** to pyridazine ring can be achieved by heating in acid media. When compounds **8** were irradiated in microwave oven at 180W and 80° C for 35 min, acetamides **10** were synthesized in 85 - 86% yields. Irradiation of **10** for a shorter period (12 min) at 90 W and 80° C led to the pyrazine **9** in 87 % yield. Synthesized compounds **7** and **8** were screened on their antibacterial activity against G- bacterial taxon *Escherichia coli*, CCM 7929 and G+ bacterial taxon *Micrococcus luteus*, CCM 732 compared to the standard 6-aminopenicillanic acid (6-APA).

BIOLOGICAL ACTIVITY

Compound	MIC (mM)	
	Escherichia coli	*Micrococcus luteus*
6-APA	3.84	5.12
7b	16.38	>20.48
7c	10.24	15.36
8a	5.12	5.12
8b	10.24	>20.48
8c	**2.56**	**1.92**

ACKNOWLEDGEMENTS

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57. Effect of various organic amendments on soil parameters of Central European Cambisol

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INTRODUCTION

Organic amendments (compared to mineral fertilizers) increase soil quality, nutrient content, and biological diversity and abundance in soil, and consequently plant health and crop yield and quality. Amendments derived from organic waste materials by pyrolysis (i.e. biochar) or by modification of conventional farmyard manure (via mineral additive to manure fermentation) represent an efficient alternative to traditional livestock waste-derived fertilizers. These types of fertilizers may exert an enhanced (compared to the conventional manure) positive effect on the soil organic carbon, organic matter degradation and the content of soil microorganisms. Field-scale experiments were carried out at arable land (Cambisol) during the cropping season 2014-2017.

MATERIALS AND METHODS

The Cambisol soil was tested in a 3-year small-scale plot field experiment in temperate zone lowland area. The experiment consisted of the application of three soil treatments - conventional manure (CM, 50 t/ha), mineral-based activator-treated manure (MBA-CM, 50 t/ha), biochar (BA, 15 t/ha) - and the mineral fertilizer (NPK) as a control. Biochar was added at the start of the experiment while manure was added every year. The experimental area was divided as follows: 3 small-scale-plots (10×10 m) per each of 4 variants of soil amendment (12 small-scale-plots overall). At the end of 3rd crop season (2017), 3 spatially independent mixed topsoil subsamples (depth 0–15 cm) from each experimental variant were collected by a soil drill at five spots and mixed in a plastic sampling bag.

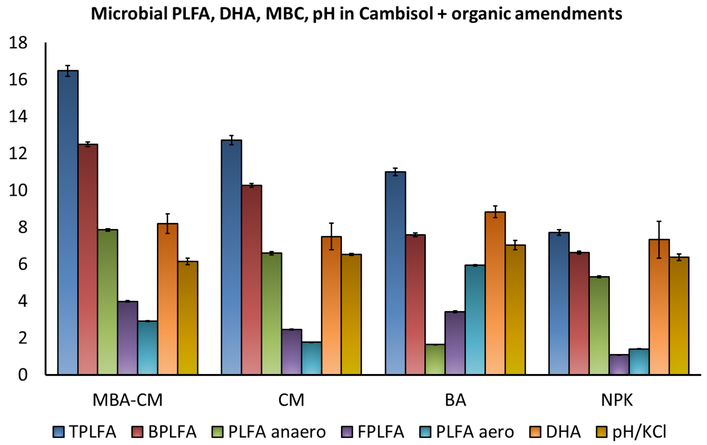
AIM

The aim was to answer these questions:

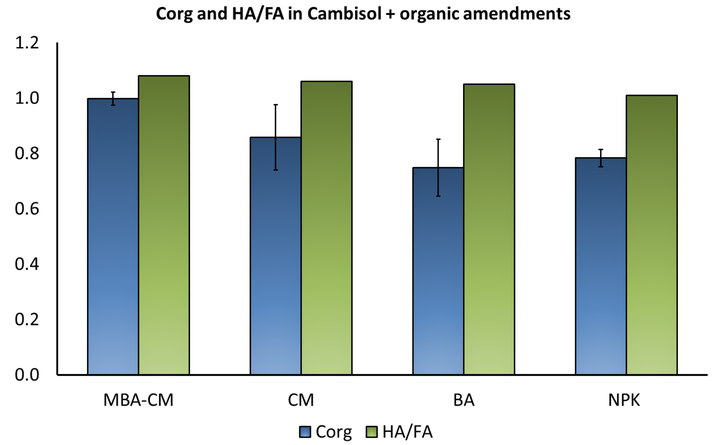
a) Can supplements with biochar improve soil content of organic carbon, microbial biomass and related properties comparably to the conventional manure amendment?

b) Does supplement of fresh manure with mineral-based activator improve the properties of product for enhancement of its positive effect on the soil organic carbon, microbial biomass and related properties as compared to the conventional manure?

RESULTS



Microbial phospholipic fatty acid content (PLFA) in soil (nmol/g of soil dry weight, d.w.): total (TPLFA), bacterial (BPLFA), anaerobic bacteria (PLFA anaero), fungal (FPLFA), strictly aerobic bacteria (PLFA aero); soil dehydrogenase (DHA, µg TPF/g d.w./h); pH/KCl. Mean values (n = 3) ± standard error of mean (SEM) = error bars.



Soil organic carbon (Corg, % d.w.); humic to fulvic acid ratio (HA/FA). Mean values (n = 3) ± standard error of mean (SEM) = error bars

CONCLUSIONS

All four experimental variants exerted significant differences in pH, dehydrogenase activity (DHA), organic carbon content (Corg), humic/fulvic acid (HA/FA) ratio, and the total, bacterial and fungal phospholipidic fatty acid content. The highest microbial abundance (+114%), Corg (+46%), and HA/FA (+7%) compared to NPK was detected in the soil amended with MBA-CM. Variable beneficial effect (compared to NPK fertilized) of different organic amendments on quality parameters of the most abundant soil type in the Czech Republic was evidenced. Mineral-based activator of biological transformation for improved effect of produced cattle manure on soil quality was valuated.

ACKNOWLEDGEMENTS

This research was supported by the projects FCH-S-23-8297, LTI20007 of Ministry of Education, Youth and Sports of the Czech Republic, and by the Ministry of Agriculture of the Czech Republic within the institutional support MZE RO1223 (Agricultural Research, Ltd. Troubsko) and MZE-RO1723 (Agrovyzkum Rapotin, Ltd.) in the frame of the Long Term Conception of the Development of the Research Organization.

ACCUMULATION OF METALS BY CELERY AND MAIZE SEEDLINGS UNDER HYDROPONIC CONDITIONS: THE ROLE OF TRANSPIRATION AND METAL SPECIATION

ANS 2023

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INTRODUCTION

Heavy metals and radionuclides pollution has attracted special attention among the scientific community due to their huge abundance, toxicity, persistent nature, and the tendency for accumulation. Metals uptake by crops have been demonstrated to be highly variable and depends on many factors in addition to the metal of interest. Chemical speciation of metals is an important parameter in environmental chemistry because ecotoxicity, mobility and accumulation of metals in plants are affected by their chemical forms.

MATERIALS AND METHODS

Seeds of celery and maize (SEMO, Inc., Czech Republic) were germinated and pre-cultivated in pots filled with granulated perlite as an inert carrier and watered with 25% Hoagland medium (*HOAGLAND, D.R.: Optimum nutrient solution for plants. Science, 52, 1920, p. 562-564*). After 6 weeks of plant pre-cultivation, seedlings were transferred into Erlenmeyer flasks with a cover to protect plant roots against lights and cultivated for 8 days in a 50 – 150 cm^3 (according to plant) Hoagland medium (HM) containing $ZnCl_2$, $CoCl_2$, $CdCl_2$, CsCl, or $SrCl_2$ and spiked with $^{65}ZnCl_2$, $^{60}CoCl_2$, $^{109}CdCl_2$, $^{137}CsCl$, or $^{85}SrCl_2$ (EUROSTANDARD, Czech Republic). In time intervals, samples of nutrient solution were taken and ^{65}Zn, ^{60}Co, ^{109}Cd, ^{137}Cs, or ^{85}Sr radioactivities were measured by gamma-spectrometry. At the end of the experiments, plants were harvested, roots were carefully rinsed in deionized water and incorporated radioactivity in roots, stems or leaves was measured. The radiometric analysis in terms of volume or specific radioactivity of ^{65}Zn, ^{60}Co, ^{109}Cd, ^{137}Cs, or ^{85}Sr in the samples of nutrient solutions or individual parts of plant was realized using a well type NaI(Tl) scintillation gamma-spectrometer 54BP54/2-X or 76BP76/3 (Scionix, Netherlands or Envinet, Czech Republic) and the data processing software ScintiVision-32 (Ortec, USA). The qualitative and quantitative proportion of the ion forms of Zn, Co, Cd, Cs, or Sr in nutrient solutions were calculated by the software MINEQL+ ver. 4.62 (Environmental Research Software, USA).

AIM

The aim of this work was to evaluate the accumulation of Zn, Co, Cd, Cs, or Sr by seedlings of celery (*Apium graveolens* L. var. *rapaceum* Mill.) and maize (*Zea mays* L. convar. *saccharata* Koern.) grown in diluted Hoagland medium (HM) spiked with $^{65}ZnCl_2$, $^{60}CoCl_2$, $^{109}CdCl_2$, $^{137}CsCl$, or $^{85}SrCl_2$ and the effect of transpiration and metal speciation on these processes. Chemical speciation of studied metals and radionuclides was predicted using modelling software MINEQL+.

RESULTS

The kinetics of Cs^+ ions accumulation by maize plants had a linear pattern and correlated with the transpiration rate. The accumulation of Zn^{2+}, Co^{2+}, Sr^{2+}, and Cd^{2+} ions by maize and celery plants did not show a linear course identical to that of transpiration. In addition, during 8 d of cultivation, the volumetric activity of ^{65}Zn, ^{60}Co and ^{109}Cd solution uptaken by the plant was higher than the volumetric activity of the HM. This indicates that in addition to the metals uptake by the transpiration stream, the adsorption and selective membrane transport of metals are also taking place. Root-to-shoot translocation (TR) of the metals decreased in the order: Cs > Sr > Zn > Cd > Co. Complexing agents, ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA), in the HM reduced Co uptake by the root system of celery (Fig. 1A). In the presence of an equimolar amount of NTA to $CoCl_2$ (10 µmol/dm^3), a 5-fold lower amount of Co uptaken by the plant in the comparison with celery plants cultivated without the presence of NTA. In the case of Sr and Cd, this effect was not observed. However, a positive effect of EDTA on Cd translocation and NTA on Co translocation was observed. In the presence of an equimolar amount of NTA to $CoCl_2$ (10 µmol/dm^3), Co translocation from celery roots to the aerial parts increased 3-fold compared with the control (Fig. 1B). Equimolar amounts of EDTA to $CdCl_2$ (10 µmol/dm^3) caused that Cd translocation was increased 1.4-fold compared with the control. The presence of EDTA, NTA, and citrate (CA) had no significant effect on Sr translocation from celery and maize roots.

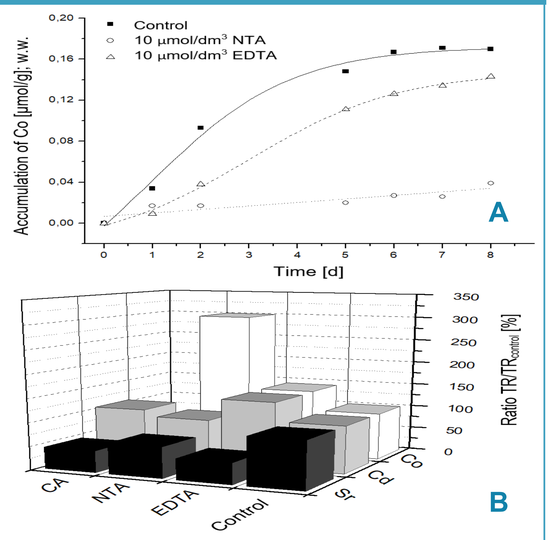

Fig. 1: Comparison of the accumulation of Co (**A**) and root-to-shoot translocation of Co, Cd or Sr by celery seedlings cultivated in 25% HM containing EDTA, NTA or CA in equimolar amounts to $CoCl_2$, $CdCl_2$ or $SrCl_2$(10 µmol/dm^3).

CONCLUSIONS

Based on the results obtained, it can be suggested that in addition to the effect of plant species the uptake and accumulation of metals by plants will be strongly dependent on the type of metal, when monovalent metals (e.g. Cs) will be uptaken mainly by activity of transpiration stream. In this comparison and in addition to the transpiration stream, divalent metals (Zn, Co, Cd, and Sr) were uptaken more intensively and by processes of adsorption and selective transport across the membranes. Speciation analysis using MINEQL+ software revealed that in the case of Co and Cd, but not for Sr, in the presence of EDTA, NTA, and citrate, the proportion of their bioavailable M^{2+} ionic forms in the HM decreased significantly in favour of $[M\text{-}NTA]^-$, $[M\text{-}EDTA]^{2-}$, or $[M\text{-}Cit]^-$ complexes. This fact led to a change in the intensity of metal uptake by roots and their translocation to the aboveground parts.

ACKNOWLEDGEMENTS

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VORTEX-ASSISTED LIQUID-LIQUID MICROEXTRACTION BASED ON NEW FLUOROPHORE FOR THE DETERMINATION OF ANIONIC SURFACTANTS IN WATER SAMPLES

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INTRODUCTION

Sodium dodecyl sulfate (SDS) is an anionic surface-active substance that, due to its specific physico-chemical properties, is used in many cleaning, hygienic, industrial and pharmaceutical products, which results in its accumulation in water courses and can have an adverse effect on various living organisms, especially aquatic organisms. Spectrophotometric, chromatographic, and potentiometric methods are the most common methods for SDS and anionic surfactants determination. The methylene blue extraction-spectrophotometric method is considered standard. The main disadvantage of the method is the large amounts of hazardous extractant chloroform used. Microextraction methods are environmentally friendly and allow reducing the consumption of organic solvent, samples, and at the same time increasing the enrichment factor and sensitivity of the analysis and simplifying and speeding up the determination of the target analyte in the samples. Despite all these advantages of microextraction methods, there are few publications in the literature about microextraction combined with fluorescence detection of SDS.

In this study, a new green, ultrasensitive and selective method based on vortex-assisted liquid-liquid microextraction (VALLME) is presented for the spectrofluorimetric determination of SDS using the new fluorophore 3,3'-diethyloxadicarbocyanine iodide (DODCI).

AIM

The aim of the work was to develop a microextraction method combined with fluorescence detection of SDS based on experimental and theoretical studies that would meet the requirements of green analytical chemistry.

MATERIALS AND METHODS

Water samples were filtered and diluted before determination. The pharmaceutical tablet was first finely crushed in a chafing dish and then dissolved in ultra pure water using an ultrasonic water bath. The tablet sample was then filtered and diluted. The prepared solutions were mixed using a vortex for 15 s at a speed of 1200 rpm. After centrifugation at 2000 rpm for 2 min, 60 µL of the extraction phase was collected by micropipette into a fluorescence microcuvette and the fluorescence intensity was measured in the wavelength range of 600-800 nm at an excitation wavelength of 640 nm with a peak around 668 nm using a luminescence spectrophotometer.

RESULTS

The principle of the method developed by us consists in the reaction of SDS with DODCI at pH 3, followed by VALLME of the formed ionic associate (IA) into the micro-volume organic phase containing n-amyl acetate.

The VALLME method is ultrasensitive and enables fluorescence detection in the area of measurement of trace amounts of SDS. The linearity of the calibration dependence was observed in the concentration ranges of SDS from 0.30 to 2.60 µg L^{-1} (R^2 = 0.9980). The limit of detection (LOD) were 0.10 µg L^{-1}. The accuracy and correctness of the proposed method was verified during two consecutive days with RSD values of 2.6-3.7% and recovery of 93.5-107.9%.

The system showed high selectivity towards SDS in the presence of at least 200-fold higher concentrations of NO_3^- 500-fold of F^-, Br^-, HPO_4^{2-}, SO_4^{2-} and 1000-fold of CO_3^{2-}; SO_4^{2-}; Cl^-; Mg^{2+}; Ca^{2+}, which do not interfere with the determination of SDS under optimal conditions.

To demonstrate the practicality of the proposed procedure, a method was developed and applied to the determination of SDS in real water samples and pharmaceuticals. Satisfactory recovery data were obtained, ranging from 93.1 to 109.0%, with a relative standard deviation of 2.2 to 4.9%.

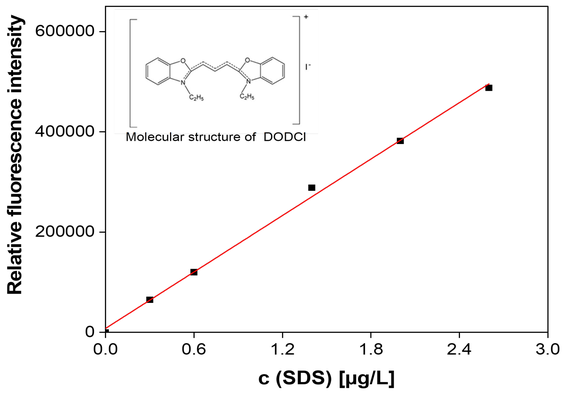

Figure 1: Calibration plot for SDS.
c(DODCI) = 5 x 10^{-6} M; pH 3; n-amyl acetate = 70 µL.

Table 1. Analytical parameters of VALLME method.

Extragent (n-amyl acetate), µL	70	500
Linear range, µg L^{-1}	0.30-2.60	3.0-14.0
Calibration equation	y = 187680x + 7905	y = 28701x + 15109
R^2	0.9980	0.9930
LOD, µg L^{-1}	0.10	0.90
LOQ, µg L^{-1}	0.30	2.90
RSD, %	2.0-5.5	2.2-4.9
Recovery, %	93.8-107.5	93.1-109.0
Enrichment factor	87	10

Table 2. Application of the suggested method to the determination of SDS in real samples (n=5, P=0.95).

Sample	SDS concentration, mg L^{-1}		RSD, %	Recovery, %
	Added	Found		
Tap water	-	-	-	-
	0.015	0.016±0.001	3.9	106.7
	0.030	0.029 ±0.001	3.9	96.7
River water	-	-	-	-
	0.015	0.014±0.001	4.9	93.1
	0.030	0.029±0.001	3.5	96.7
Water after washing	-	6.8±0.2	2.8	-
	3	10.0±0.3	2.5	106.7
	6	12.6±0.4	2.4	96.7
Water after the second rinsing of laundry	-	80.4±2.4	2.4	-
	30	109.6±3.9	2.8	97.3
	60	145.8±3.9	2.2	109.0
Herpesín (aliquot = 0.5 mL)	-	22.9±0.9	3.0	-
	15	38.3±1.3	2.7	102.7
Herpesín (aliquot = 1.0 mL)	-	22.3±0.8	3.0	-
	7.5	30.0±1.3	3.5	102.7

CONCLUSIONS

The obtained results show that the VALLME method coupled with fluorescence detection is ultrasensitive, selective, simple and reliable in the detection of SDS with requirements for green analytical chemistry and has great promise for environmental applications.

ACKNOWLEDGEMENTS

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AVENANTHRAMIDES AS POTENTIAL PHARMACEUTICALS AND THEIR OCCURENCE IN DIFFERENT GENOTYPES OF *AVENA* SPP.

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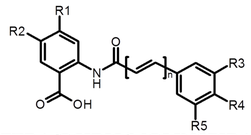
INTRODUCTION

AVENANTHRAMIDES (anthranilic acid amides) are unique phenolic alkaloids found exclusively in oats. Recently, various bioactivities have been found in avenanthramides as an antioxidant activity, anti-inflammatory, anti-atherogenic and anti-proliferative effect. The general formula of main avenanthramides A, B and C (AVNs), including categorization by Collins* and Dimberg´s** modified system is presented [1, 2].



Main avenanthramides		n	R_1	R_2	R_3	R_4	R_5
A*	2p**	1	H	OH	H	OH	H
C	2c	1	H	OH	OH	OH	H
B	2f	1	H	OH	OCH_3	OH	H

PETRA/OSIRIS/MOLINSPIRATION (POM) analysis is an efficient bioinformatic approach used to assess the basic physical-chemical parameters of the molecules and predict bioactivity, toxicity, drug-likeness parameters [3]. The title of this approach consists of the software PETRA, OSIRIS, and the free online tool MOLINSPIRATION. PETRA (Parameter Estimation for the Treatment of Reactivity Applications) is a program package comprising various empirical methods for calculating basic physicochemical properties in organic molecules. In the modified POM analysis is PETRA calculation repleaced by SWISS ADME calculations.

MATERIALS AND METHODS

Hundred oats varieties were cultivated in the experimental area of the Research and Breeding Station at Vígľaš-Pstruša of the National Agricultural and Food Centre, Research Institute of Plant Production Piešťany, Slovak Republic. The oats varieties obtained from the harvest in year 2019, 2020 and 2021. The extract preparation were carried out by optimal conditions, the extraction time was adjusted by ultrasound-supported extraction (15 min cycle).

HPLC was performed on a Waters instrument equipment with Separations Module e2695, DAD detector 2998 and software Empower 3. Avenanthramides were separated on a reversed phase C-18 (Cortecs 4.6 x 100 mm, 2.7 µm particle size) by step gradient: 0-2 min. 12% B; 2-16 min. 12-28% B; 16-19 min. 100% B. Component A of the mobile phase was 0.1% aqueous solution of formic acid, component B was acetonitrile with 0.1% formic acid, flow 1ml/min, column temperature 35°C, UV detection at 320 nm, UV spectra 210-400 nm. The detected peaks were identified by comparing their retention times and ultraviolet (UV) spectra with the avenanthramide standards. Each experiment and each HPLC analysis was performed in triplicate.

High resolution mass spectrometry (HRMS) confirmed the presence of avenanthramides AVN A (2p), AVN B (2f) and AVN C (2c), three major avenanthramides in selected oat genotype: CDC Mistrel.

The structures of 42 avenanthramides were calculated *in silico* in according with particular protocols to these via public accessible calculating tools.

AIM

Main goal of this work was to introduce the Aveanthramides as new, perspective group of bioactive compounds, by the effect comparable with polyphenols.

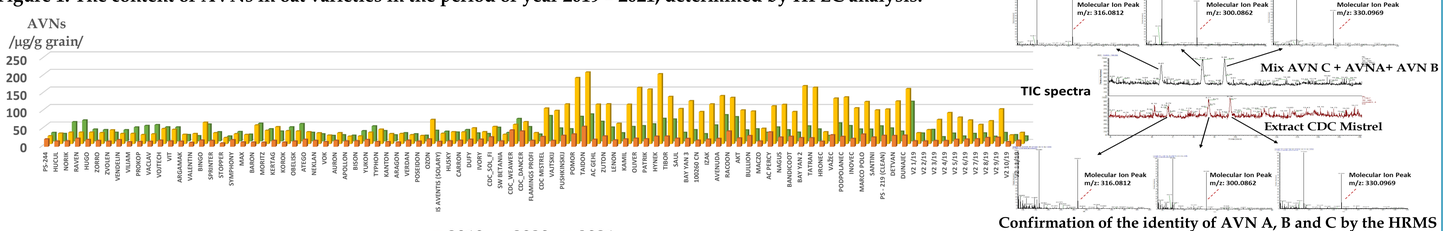
Partial goal No. 1 was to find a suitable biological source of these substances. We investigated the content of avenanthramides in the grain of different oat genotypes, grown and harvested in Slovakia in the Vígľaš-Pstruša location on isolated fields.

Partial goal No. 2 was to subjected the structure of avenanthramides to modified POM analysis *in silico*. It was performed a modified POM analysis of 42 AVNs, described in literature using MOLINSPIRATION, SWISSADME, and OSIRIS software.

The conclusion of this study was to select particular avenanthramides, found in in the grain of different oat genotypes, mentioned in text therein before these ones, which has optimal predictions about drug likeness, ADME parameters and low toxicity.

RESULTS

Figure 1. The content of AVNs in oat varieties in the period of year 2019 – 2021, determined by HPLC analysis.



Confirmation of the identity of AVN A, B and C by the HRMS

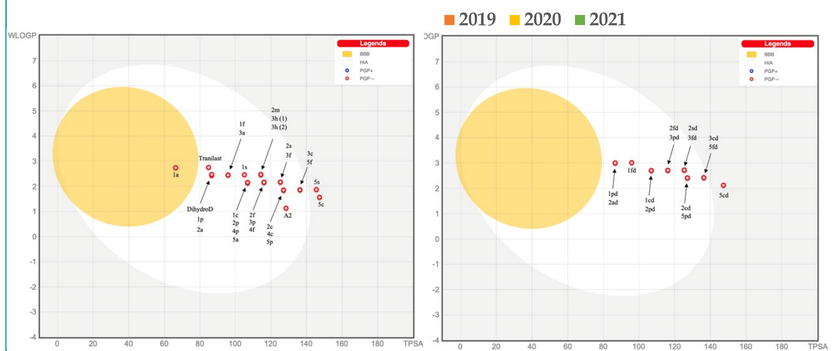


Figure 2. A diagram for AVN derivatives of 2-[(1-Oxo-3-phenyl-2-propen-1-yl)amino]benzoic acid (upper chart) and 2-[[(2E,4E)-5-phenyl-1-oxo-2,4-pentadien-1-yl]amino]benzoic acid (lower chart).

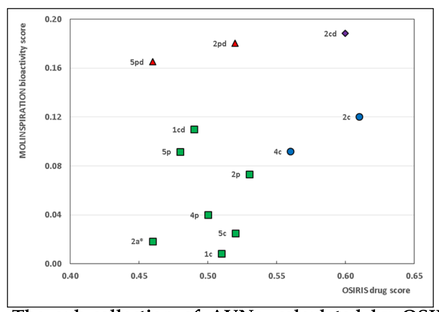


Figure 3. The sub-collection of AVNs, calculated by OSIRIS and MOLINSPIRATION. The most promising substances are marked as red triangles (+ violet rhombus) and blue circles (+ violet rhombus).

CONCLUSIONS

1. The natural compounds are still a very perspective subject of the research, aimed to discover and offer new skeletal types for new drug generations.
2. Avenanthramides are very perspective group of specific plant metabolites, found mainly in oats, currently intensively studied, published, and expressing wide variety of promising biological effects.
3. The higher content of AVNs (AVN A, B, C) was found in following varieties: TAIDON, HYNEK, POMOR, BAY YAN 2, TATRAN, OLIVER and DUNAJEC.
4. Modified POM analysis reveals significant differences among individual AVNs; particularly AVNs 4c, 2c (AVN C), and 2cd are the most favorable candidates with optimal drug likeness parameters, ADME properties and low toxicity.
5. Avenanthramide 2c (AVN C) was found to be significantly present in evaluated oat varieties, thus especially this particular avenanthramide could be a one of final target of breeding attempts.

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INFLUENCE OF SURFACTANTS ON ARSENIC BIOLEACHING FROM MINING WASTES

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INTRODUCTION

Heavy metal contamination of the environment is particularly problematic in former mining areas. In Poland, one of the old arsenic-gold mining and processing centres is Złoty Stok in the southwest of the country. Ore mining and processing generated large amounts of waste materials rich in As, such as mine waste rock.

The immobilisation of bacterial cells on the mineral surface promotes bio-oxidation, which under favourable conditions can cause acid mine drainage and arsenic release. Therefore, it is important to control these phenomena. One method is to change the surface properties of the solid by adsorption of surfactants. Similar modifications are used in mineral processing, such as flotation or flocculation. Therefore, bioleaching experiments were performed using pure and modified arsenic-bearing waste (after contact with cationic and anionic surfactant), to define conditions under which arsenic release can be inhibited.

MATERIALS AND METHODS

Mineral material: Arsenic-bearing waste was taken from a large stockpile of mining waste deposited in the area of the backfilled Jan shaft in Złoty Stok, Poland (particle size 1-1.25 mm).

Surface modification: The mineral was conditioned with 0,1 M cetyltrimethylammonium bromide CTAB, and sodium dodecyl sulfate, SDS (Sigma-Aldrich), for 24h.

Bioleaching was carried out in columns (68 x 4.5 cm) with 50 g of pure or modified arsenic waste. A consortium of autochthonic, acidophilic microorganisms, grown on 9K medium was used (inoculum 10% v/v, pH 2.0). The metal concentration was determined through the inductively coupled plasma-optical emission spectrometry technique (Agilent 5110 ICP-OES Agilent Technologies, Australia).

Zeta potential: The surface potential of leaching residues (1-1.25 mm) was measured using Surpass3 analyzer (Anton Paar, Austria), and solid by-products (fine particles) was measured with the Zetasizer 2000 (Zetasizer, Malvern, United Kingdom) at a constant ionic strength of 10^{-3} M NaCl, pH 2.0.

AIM

- Surface modification of arsenic-bearing waste to inhibit the metal release into the environment.
- Investigation of the surface properties of bacteria, arsenic waste, and secondary products by monitoring zeta potential in oder to explain bacteria-mineral-surfactant interactions in bioleaching.

RESULTS

Table 1. Elemental analysis of arsenic waste

Element	Amount [g/kg]
Al	45.16
As	16.38
Fe	58.06
Pb	0.196
Si	239.6
Zn	0.054

Table 2. Zeta potential values of samples

Sample name	Zeta potential [mV]
Bacteria	4.70
As waste	- 0.45
As waste + CTAB	5.71
As waste + SDS	- 8.24
Control	4.09
BIO	13.8
BIO (CTAB)	11.8
BIO (SDS)	14.1
BIO (secondary product)	26.6
BIO (CTAB) (secondary product)	24.3
BIO (SDS) (secondary product)	24.8

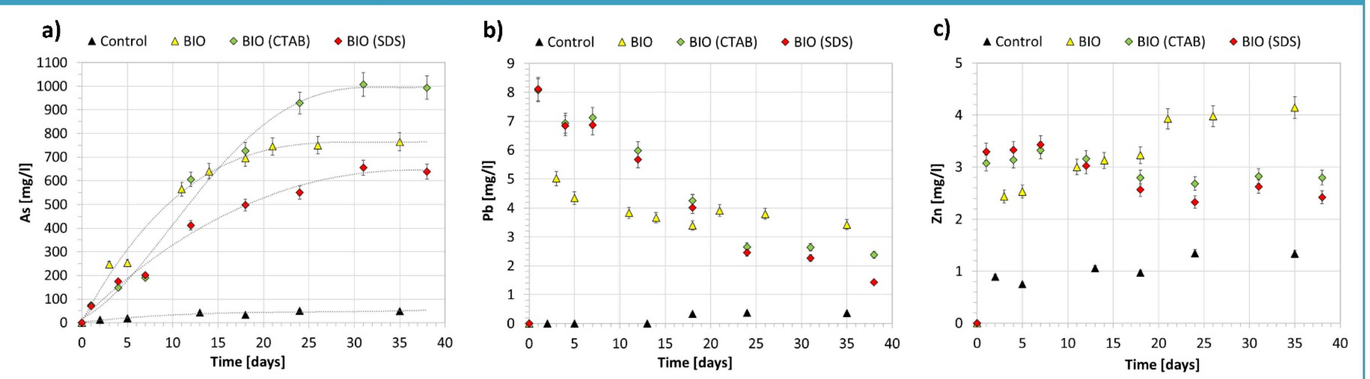

Figure 1. Changes of **a)** As, **b)** Pb, and **c)** Zn concentration within time; Control – proces without bacteria; BIO – bioleaching of aresenic waste; BIO (CTAB) – bioleaching of arsenic waste after contact with CTAB; BIO (SDS) – bioleaching of arsenic waste after contact with SDS

Figure 2. Mineral particles of arsenic waste and secondary products after bioleaching

Table 3. Elemental analysis of secondary products

Element [g/kg]	Secondary product		
	BIO	BIO (CTAB)	BIO (SDS)
As	8.984	4.201	5.194
Fe	240.2	326.7	335.6
Pb	0.356	0.381	0.331
S	108.8	126.9	122.8

CONCLUSIONS

- **The presence of cationic surfactant (CTAB) on the mineral waste surface increased the arsenic removal**, and the adsorption of anionic surfactant (SDS) inhibited metal release.
- **The presence of surface-active compounds promoted lead bioextraction**, but after one day of the process, a systematic decrease in concentration was observed. Probably due to the precipitation of lead sulfate. In the case of zinc, its concentration for modified arsenic waste was higher within the first seven days and then decreased slightly until the end of the process.
- **The adsorption of surfactants caused changes in the surface properties of mineral waste.** The arsenic-bearing surface charge became more negative after contact with SDS due to the presence of negatively charged sulfate polar heads of surfactant. When mineral waste was conditioned with CTAB, The SiO^- anion present on the waste surface interacted with the CTAB-charged cation head group (N^+-$(CH_3)_3$) resulting in a positive zeta potential.
- **As a result of the bio-oxidation, precipitation of the secondary product occurred leading** to positive zeta potential of bioleaching residues.

ACKNOWLEDGEMENTS

This activity was realized within grant no. 2021/43/D/ST10/02784, financed by the National Science Centre, Poland

ANS 2023

Utilisation of Microextraction Technique for Preconcentration and Determination Selected Xenobiotics in Fruit using GC-MS

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INTRODUCTION

The European Commission specified the maximum residual limit (MRL) of 10 µg/kg of pesticide residue content in cereal-based foods and baby foods for infants and young children.

Requirements on sample preparation method:

- Multirezidual method
- Recoveries close to 100 %
- High cleaning efficiency
- Good precision
- Good ruggedness
- Low limits of quantification (LOQs)
- Economic, ecologic and safety demands (Cheap, Easy, Fast, Less harmful solvents, Low amount of solvents.

MATERIALS AND METHODS

GC-MS instrumentation and conditions

Agilent 6890N GC coupled to 5973 MSD, Programmed temperature vaporizer (PTV): 40 °C (0.2min), 400 °C/min up to 300 °C (2 min), 400 °C/min up to 350 °C (5 min)
Injection volume: 2 µl
CP-Sil 8 CB chromatographic column, 15 m x 0.15 mm I.D. x 0.15µm
Carrier gas: He, constant flow 1.2ml/min
Oven temperature program: 60 °C (1.75 min), 60°C/min to 150 °C, 30°C/min to 300°C (3 min)
Electron impact (EI) ionization, SIM mode, dwell time 10 ms

Sample preparation method

The commercial TwisterTM stir bar for sorptive extraction obtained from Gerstel (Mullheim an der Ruhr, Germany) was used. It consists of a 1.0-cm-long glass-encapsulated magnetic stir bar externally coated with PDMS.
The volume of sample (5 ml), the extraction time was optimized (135 min).
The desorption time (40 min), desorption volume (195 µl in ACN).

AIM

The main objective of this work was selection and optimization of selected parameters for determination of 11 pesticides in strawberries using the environmentally friendly extraction technique known as stir bar sorptive extraction (SBSE), followed by liquid desorption in a solvent and fast capillary gas chromatography–mass spectrometry. Optimization of the method was obtained.

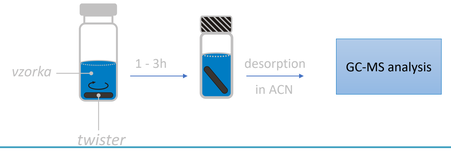
The other aim of the work was determination of selected pesticide residues in real samples from Slovak market using optimalised SBSE in combination with GC-MS.

Optimization of the extraction procedure

- optimization of sample volume (5 ml),
- optimization of extraction time (45min, 90 min, 135 min, 180min, 225 min, 270 min),
- optimization of desorption time (10min, 20min, 30min, 40 min, 50min, 60min),
- Optimization of effect of ionic strength on the extraction process (0g, 0.1g, 0.5g, 1g,2g of NaCl)

Selected extraction conditions:

- sample volume - 5 ml
- temperature of extraction - 25°C
- constant stirring – 700 rpm
- extraction time - 135 min
- desorption time - 40 min
- desorption volume - 195 µl

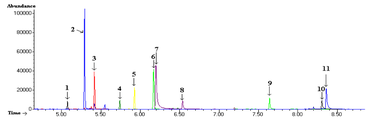


Tab.1 Limits of detection (LOD), limit ofquantification (LOQ) and coefficient of determination (R^2) in strawberry matrix after SBSE sample preparation.

Pesticide	LCL [ng/ml]	RSD [%]	LOD [ng/ml]	LOQ [ng/ml]	R^2
Hexachlorbenzene	0.5	11.9	0.192	0.641	0.9880
Diazinon	0.25	28.8	0.04	0.16	0.9370
Antracen	0.001	33.36	0.0003	0.001	0.9910
Chlorpyrifos	2.5	14.6	0.26	0.89	0.9780
Cyprodinil	0.001	17.5	0.00004	0.00014	0.9860
Penconazole	0.005	12.6	0.0002	0.00061	0.9540
Fludioxonyl	0.25	16.4	0.076	0.253	0.9920
Pyriproxyfen	0.1	6.98	0.0096	0.032	0.9830
Boscalid	0.05	23.7	0.01	0.035	0.9920
Pyridalyl	0.005	9.6	0.0038	0013	0.9800

Tab.2 Concentration in [µg kg $^{-1}$] of pesticide residues determined in real samples (strawberries).

Pesticide	Pesticide concentration (µg/kg)		
	Andalusia	Hungary	España
Hexachlorbenzene	-	-	0.311
Antracen	0.120	0.145	-
Penconazole	0.070	0.036	-
Fludioxonyl	0.029	0.019	0.017
Boscalid	-	36.880	0.224



1 Hexachlorbenzene
2 Diazinon
3 Antracen
4 Heptachlor
5 Chlorpyrifos
6 Cyprodinil
7 Penconazole
8 Fludioxonyl
9 Pyriproxyfen
10 Boscalid
11 Pyridalyl

Chromatogram of the tested pesticides analysed with fast GC-MS in SIM mode in ACN extract using SBSE extraction.

CONCLUSIONS

Time of extraction, sample volume, stirring speed and the addition of different amounts of NaCl were evaluated to reach the best overall extraction and analytical conditions. The factor extraction temperature has a significant influence on the SBSE efficiency, but a high temperature reduces the lifetime of the PDMS phase, so in this work, the extraction temperature was set at 25 ◦C.

The developed procedure SBSE is simple, sensitive, reproducible, and economical for the determination of selected 11 pesticides in strawberries.

Optimised SBSE in combination with GC-MS was used for determination of tested pesticides in 3 real strawberry samples (from Andalusia, Hungary, Espaňa).

ACKNOWLEDGEMENTS

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DETERMINATION OF BIOGENIC AMINES IN FOODS BY HPLC

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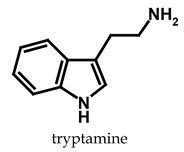
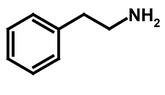
INTRODUCTION

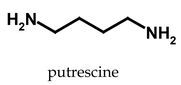
Biogenic amines (BAs) are biologically active substances, which occur naturally in a variety of organisms. They are organic nitrogen compounds with low molecular weight and of the various characteristic structures containing amino groups. In small amounts are found in living organisms, including humans. BAs play essential role in physiology, including normal cell growth and development. They are a source of nitrogen for the synthesis of hormones, alkaloids, nucleic acids and proteins. In living organisms they arise by decarboxylation of amino acids, as well as amination and transamination of aldehydes and ketones. Their amount increases in direct proportion with microbial fermentation. Interests are mainly in terms of medicine, but mainly food. Since biogenic amines are biologically active substances with significant effect on human health, it is necessary to control the concentrations of these substances in foods.



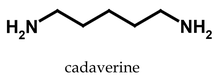
tryptamine



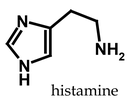
phenyletylamine



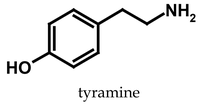
putrescine



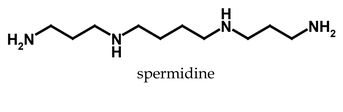
cadaverine



histamine



tyramine



spermidine

MATERIALS AND METHODS

Reagents: tryptamine 98% (TRY), phenylethylamine 99% (PHE), 1,4-diaminobutane dihydrochloride 99% (putrescín – PUT), 1,5-diaminopentane 98% (kadaverín – CAD), histamine dihydrochloride 99% (HIS), tyramine hydrochloride 99% (TYR), spermidine trihydrochloride 99% (SPD), 1,7-diaminoheptane 98% (internal standard Derivatization reagent dansylchloride (c = 5 mg ml^{-1}), (from ACROS).

Sample preparation: 10g of meat sample was homogenized in 90 ml of 10% TCA for 1 min. Followed filtration through a membrane filter. The vegetable and fruit samples were juiced and mixed with 5% TCA in a 1: 1 ratio and subsequently filtered through a membrane filter. Samples of the cheeses (10 g) were gently shaken and homogenized in 5% TCA for 15 min and also filtered.

Derivativatization: To 800 µl of the sample was added dansyl chloride 500 µl and sodium carbonate solution 500 µl into a 50 ml glass flask. In the method of the internal standard diaminoheptane (DAH) solution was added, 50 µl. After mixing, the flask was incubated in water bath at 70 ° C for 15 min. The sample was cooled and concentrated by vacuum evaporation and then dissolved in 2 ml of acetonitrile. Sample was analysed by HPLC. All samples were stored at -20 ° C until analysis.

HPLC analysis: HPLC chromatograf (Waters) with DAD detector, softvare Empower 2.
Column Symmetry C-18 (Waters, 4.6 x 75 mm, 3.5 µm) was used. Used mobile phase as follovs: methanol 32%, acetonitrile, acetic acid (0.02 mol.l^{-1}) 30%, izocratic system, Flow-rate of 1 ml min^{-1} Detection at 254 nm 20 ml of sample was injected, Temperature was kept at 30 °C.

RESULTS

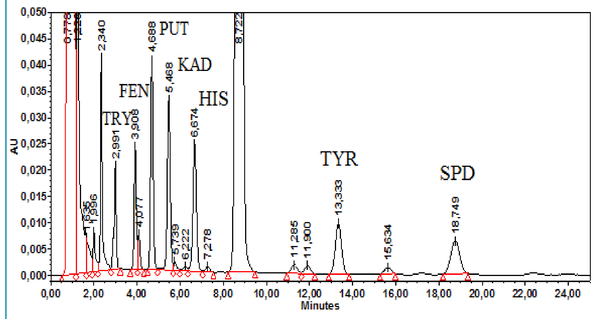


Fig. 1 Chromatogram of standard sample (10 µg/ml)

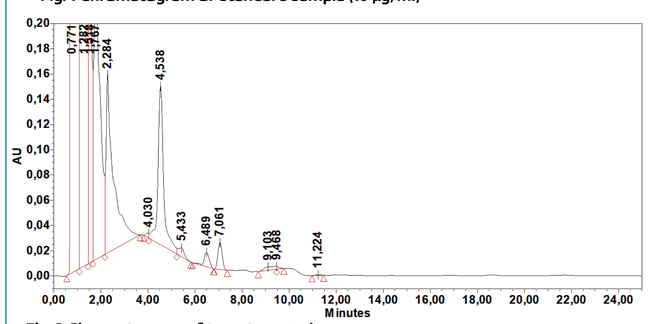


Fig. 2 Chromatogram of tomato sample

Tab. 2 Recovery of spiked samples of meat

BA	Added in mg to 10 g of sample	Recovery in %
Tryptamine	0,1	81
Phenyletylamine	0,1	87
Putrescine	0,1	96
Cadaverine	0,1	98
Histamine	0,1	91
Tyramine	0,1	88
Spermidine	0,1	96

Tab. 1 Analytical parameters for determination BA (concentrations from 2,5 to 200 µg/ml)

Biogenic amines	RT (min)	Calibration curve	R^2
Trypthamine	2,99	y = 10097x + 12019	0,9992
Phenylethylamine	3,90	y = 12037x + 14946	0,9994
Putrescine	4,68	y = 29542x + 32271	0,9996
Cadaverine	5,46	y = 30350x + 49026	0,9976
Histamine	6,66	y = 24052x + 39281	0,9996
Tyramine	13,31	y = 14802x + 32089	0,9917
Spermidine	18,70	y = 11661x + 14553	0,9809

Tab. 3 BA in sample of ham (mg/kg), n = 3

Sample	TRY	PHE	PUT	CAD	HIS	TYR	SPD
1	1	0	0	0	1,5	0	2,1
2	2,3	0	6,2	0	0	0	1,6
3	4,3	0	0	0	12,1	0	0
4	0,0	0	1,8	3,1	0,0	1,9	2,7
5	15,2	0	0	8,1	0	7,1	6,4

Tab. 4 BA in cheese

Sample	TRY	PHE	PUT	CAD	HIS	TYR	SPD
bryndza 1	0	0	58,60	5,0	24,0	51,5	3,1
bryndza 2	0	0	3,6	12,1	3,6	19,3	2,6
bryndza 3	1,0	0	2,8	0	3,6	0	0
eidam	0	0	6,9	45,2	6,2	8,2	3,3
niva	0	0	30,6	0	0	26,2	32,3

Tab. 5 BA in vegetables

Sample	TRY	PHE	PUT	CAD	HIS	TYR	SPD
cabbage	0	0	81,2	34,5	6,1	51	17,8
Tomato	0	0	137,8	4,2	7,1	0	0
Carrot	49,9	0	158,9	0	0	0	21,9
cucumber	40,0	0	133,4	0	0	0	19,0
broccoli	36,0	0	445,6	0	10,7	0	37,6

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CONCLUSIONS

Monitoring of biogenic amines:
As a factor of quality and freshness of food.
As a factor in the biotechnological process (fermented food)
As an important factor in the field of medicine

Supported by KEGA 00UCM4/2021

EFFECT OF SOIL NUTRIENTS ON THE STABILITY OF SUPERABSORBENT POLYMERS: HYDROGEL SWELLING AND POTASSIUM RELEASE

ANS 2023

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INTRODUCTION

Drought is a critical factor that adversely affects crop production and yield stability. As the result of climate change, rainfall variability is expected to increase, leading to more frequent drought events and intense rainfall events with devastating consequences for crop productivity and the whole agroecosystem. Superabsorbent polymers (SAPs) represent a potential tool to mitigate drought stress in agriculture due to their excellent water absorption and water retention properties.

MATERIALS AND METHODS

The superabsorbent polymer (SAP) Aquaholder® Seed (PeWaS Ltd., Slovak Republic) based on acrylic acid, potassium salt of acrylic acid and acrylamide (national patent No. PCT/SK2019/050008) for agricultural applications was used to evaluate the effect of soil nutrients, especially alkali metals and microelements, on hydrogel swelling (water absorption) and the release of potassium as an important macronutrient in plant nutrition. In the experiments, hydrogels were prepared in mass concentrations of 10 mg (SAP)/10 ml or 20 mg (SAP)/10 ml of deionized water or salt solutions of KCl, NaCl, CsCl, $ZnCl_2$, $CuCl_2$, $CoCl_2$, $CdCl_2$, or Hoagland's medium (HM) of 10% or 100% of concentration strength (*HOAGLAND, D.R.: Optimum nutrient solution for plants. Science, 52, 1920, pp. 562-564*). The determination of potassium was carried out by flame photometry (ATS 200MKI, Switzerland) or scintillation gamma-spectrometry – NaI(Tl) scintillation detector 76BP76/3 (Envinet, Czech Republic). Gamma-spectrometric analysis and calculation of activity were carried out by data processing software ScintiVision-32 (Ortec, USA). Assessment of water binding (hydrogel swelling) of SAP was carried out gravimetrically (KERN ABJ 120-4NM, Germany). The statistical significance of the obtained results was analyzed on the basis of Duncan's multiple range test using the STATGRAPHICS Centurion XV ver. 15.1.02.

AIM

The aim of this work was to evaluate the effect of soil nutrients, especially alkali metals and microelements, on hydrogel swelling (water absorption) and potassium release as an important macroelement in plant nutrition. Sodium binding in the hydrogel was also studied in terms of potassium release. For these purposes, a commercially available superabsorbent polymer Aquaholder® Seed (PeWaS Ltd., Slovak Republic) designed for agricultural applications was used.

RESULTS

The studied SAP contained K in mass fraction of 12.0 %, as determined by flame photometry and also confirmed by measurement of ^{40}K isotope using scintillation gamma-spectrometry. Application of deionized water and solutions with equimolar amounts of Na, Cs, Zn, Cu, Co, and Cd to the K present in the polymer resulted in the release of K from the SAP in the amounts of 14.2%, 34.2%, 31.4%, 48.0%, 67.0%, 43.7%, and 76.2%, respectively (Fig. 1A). HM solutions of 100% or 10% of concentration strength mimicking the soil solutions were also used in this series of experiments. When 100% HM was applied, K release was 47.4% and when the SAP was treated with 10% HM solution, 17.4% of K was released from the hydrogel formed. In the case of application of solutions containing Na, sodium binding was evaluated in addition to K release. It was found that when equimolar amounts of Na and solutions of 100% HM and 10% HM were used, Na binding by the polymer was 30.8%, 22.8%, and 59.0%, respectively. In these cases, the molar ratios between the amount of K released and the amount of Na bound were 0.63, 1.44, and 0.62, respectively. Water absorption or hydrogel swelling in the presence of equimolar amounts of Na, K, Cs, Zn, Cu, Co, and Cd to the K present in the SAP or in the presence of 100% HM and 10% HM solutions in the comparison with the control (deionized water) decreased in the following order: deionized water (100%) > 10% HM (69.3%) > K (60.1%) > Cs (60.0%) > Na (57.3%) > 100% HM (18.8%) > Co (16.2%) > Cd (12.8%) > Zn (12.4%) > Cu (6.5 %) (Fig. 1B).

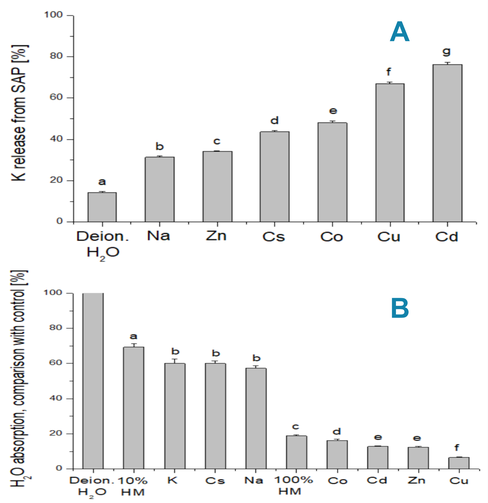

Fig. 1: Comparison of the K release from SAP (**A**) and water absorption (hydrogel swelling; **B**). Mean values from triplicate experiments with the same letter above columns are not significantly different at the $p < 0.05$ level based on Duncan´s multiple range test.

CONCLUSIONS

The results obtained suggest that the presence of soil nutrients, especially alkali metals or microelements, can cause a significant release of K from SAP and its polymeric structure as an important macroelement in plant nutrition. This will be mainly due to changes or degradation of the three-dimensional polymer network, which also leads to a reduction in the water absorption efficiency of the SAP. Separate results showed that the hydrogel is able to bind sodium, with a significant release of K in this process, and hence the replacement of K by Na in the polymer structure. On the basis of these results, it can be assumed that under real conditions ion exchange between the hydrogel and soil solution can lead to a significant release of K and absorption of other alkali metals or microelements in hydrogel formed, as well as degradation of the polymer structure in terms of its ability to absorb the water.

ACKNOWLEDGEMENTS

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ANS 2023

IRON COMPLEXES WITH DIFFERENT AMINO ACIDS

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INTRODUCTION

Iron is an essential element present in the human body obeying many functionalities [1].

The toxicity of excessive free iron and its potential role in neurodegenerative diseases have been well documented, but there is a lack of fundamental physico-chemical information dealing with the substances that can interact with free iron like **amino acids** and **neurotransmitters.**

MATERIALS AND METHODS

The aim of this work was to study the **reaction of iron compounds and with selected amino acids** and the study of the influence of experimental conditions on the composition of the product. Complexes were prepared:

- with different types of reactants,
- at varying pH values of the reaction environment
- varying values of reactant ratios.

The obtained products were characterized by elemental analysis and infrared spectrophotometry. Based on the results of the analyses, conclusions were made about the influence of different reaction conditions on the synthesis of complexes.

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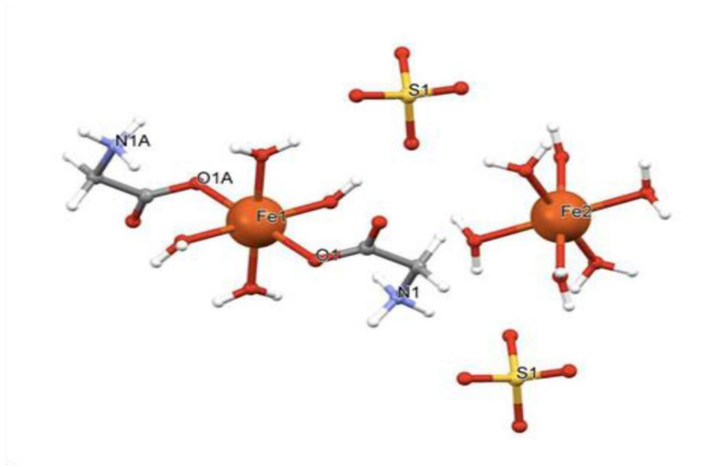
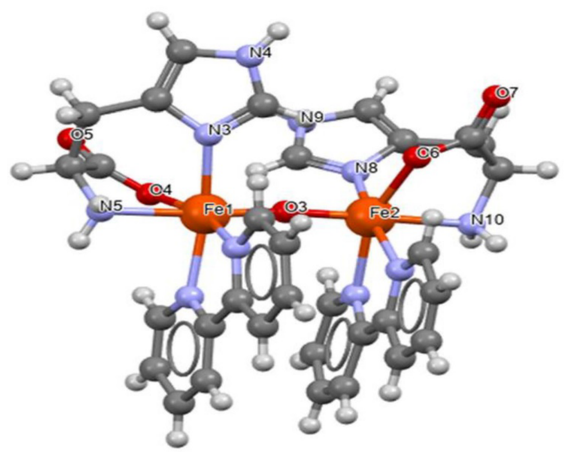
RESULTS

We studied the preparation and properties of iron complexes with amino acids **glycine and histidine**.



Obrázok 1 Molekulová štruktúra komplexu $[Fe(GlyH)_2(H_2O)_4][Fe(H_2O)_6](SO_4)_2$



Obrázok 2 Molekulová štruktúra komplexného katiónu $[\{Fe(L\text{-}his)(bpy)\}2(\mu\text{-}O)]^{2+}$

CONCLUSIONS

The amino acids, e.g. glycine glyH, can form different products from complex species with ferrous, or ferric ions showing different stability to complexes obtainable in solid state $([Fe(glyH)_2(H_2O)_4][Fe(H_2O)_6](SO_4)_2$, catena-$[Fe(H_2O)_2(SO_4)_2(\mu\text{-}glyH)Fe(H_2O)_4(\mu\text{-}glyH)]_n$) or some other product (anhydrous ferrous glycine sulfate − FeGS) as dietary supplement is formed.

The study of iron complexes formation with different amino acids has shown, that the composition of the final products are rather complicated due to redox reactions that in oxygen presence finally led to iron oxides formation [2].

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ENHANCEMENT OF HPLC METHOD FOR POLYPHENOLS DETERMINATION IN DIFFERENT PLANT SAMPLES

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INTRODUCTION

Polyphenolic compounds are ubiquitously distributed among plants, affecting the organoleptic and nutraceutical properties of fruits and vegetables but also the plant's ability to cope with environmental constraints. These metabolites have been identified as playing a role in the environmental stress responses of plants. Their detection and quantification represent a major challenge due to their low concentration levels and high-water content in tissues, a wide range of polarity, and metabolite complexity of green plant tissue. Furthermore, analyses are hampered by extractability and solubility (bound or free) of individual compounds and limited stability of the analytes. Routine analysis of phenolic compounds in plants requires a complex pre-processing of plant samples.

MATERIALS AND METHODS

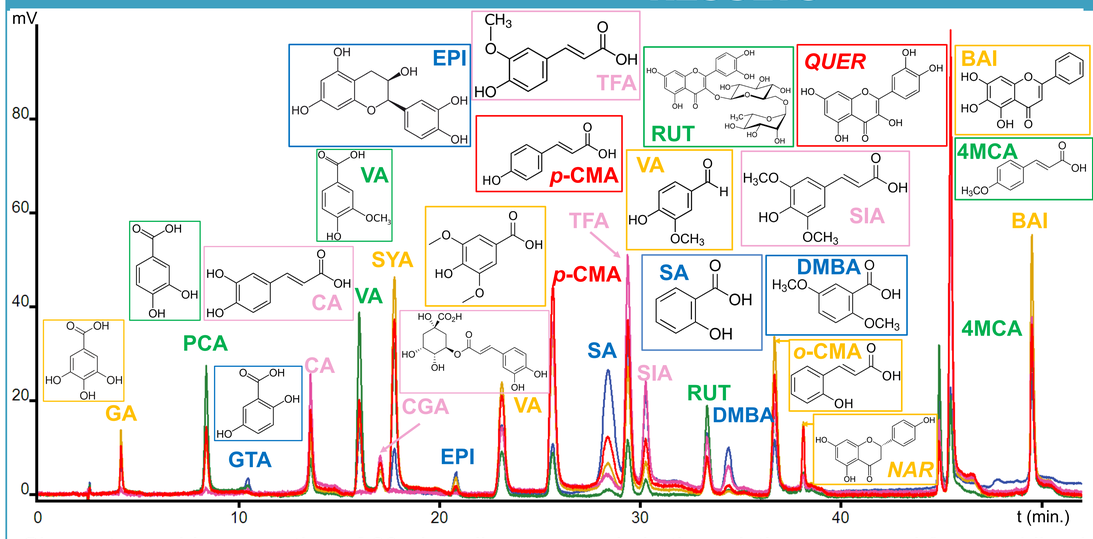
- HPLC system Young Lin 9100 with vacuum degasser, quaternary pump, column thermostat, diode array detector, autosampler operated by software Clarity v. 7.3;
- column: ReproSil-Pur 120 C18-AQ (150 x 4,6 mm, 3 µm, Dr. Maisch GmbH) with precolumn: ReproSil-Pur 120 C18-AQ (5 x 4,6 mm, 3 µm, Dr. Maisch GmbH) tempered at 35 °C;
- mobile phase flow rate 1.0 mL/min with composition:
  component (A): AcN/H_2O/CF_3COOH (95/5/0.05; v/v/v)
  component (B): H_2O/AcN (95/5; v/v) with pH=4 modified by CF_3COOH
- sample solution contained 50/50 (v/v) of both mobile phases (A) and (B) and injection volume was 5 µL;
- gradient program was set up as % increase of component (A): 0–5 min 1.5 %; in 10–17 min 7.0 %; in 25–30 min 12.0 % and in 55 min 50.0 %;
- detector (PDA) recorded wavelengths: 240, 260, 280, 290, 325, and 350 nm;

AIM

1. Increase the number of analytes in previously published HPLC method (Ranušová, *et al.*, Separations 8, 2021) from 16 to 20 phenolics by adding syringic acid (SYA), salicylic acid (SA), naringenin (NAR) and baicalein (BAI)

2. Improve the overall chromatographic performance such as:
   a) reducing the analysis time
   b) peak parameters
   c) method performance: lower LOD & LOQ
   d) increasing the column resilience

RESULTS



Elution order of analytes:
1. (GA) Gallic acid;
2. (PCA) Protocatechuic acid;
3. (GTA) Gentisic acid;
4. (CA) Caffeic acid;
5. (VA) Vanillic acid;
6. (CGA) Chlorogenic acid;
7. (SYA) Syringic acid;
8. (EPI) Epicatechin;
9. (VAN) Vanillin;
10. (p-CMA) *p*-coumaric acid;
11. (SA) Salicylic acid;
12. (TFA) *trans*-ferulic acid;
13. (SIA) Sinapic acid;
14. (RUT) Rutin;
15. (DMBA) 2,5-dimethoxybenzoic acid;
16. (o-CMA) *o*-coumaric acid;
17. (NAR) Naringenin;
18. (4MCA) 4-methoxycinnamic acid;
19. (QUER) Quercetin;
20. (BAI) Baicalein

Chromatographic separation of 20 phenolic compounds in the solution composed from mobile phase components (A) and (B) in 50/50 (v/v) ratio and concentration of all analytes c = 25 µg/mL. Figure shows overlay of chromatograms recorded on wavelengths: **240**, **260**, **280**, **290**, **325 nm**.

CONCLUSIONS

- ✓ Presented preliminary results shows fulfilment of several aims of this study:
  1.) successful enhancement of HPLC method for 4 new analytes: salicylic acid, syringic acid, baicalein and naringenin;
  2.) analysis time reduction from original 65 min to 55 min;
  3.) changes in mobile phases increased the pH level from 3 to 4, which was much „friendlier" to the column and improved also other aspects of chromatographic performance of enhanced HPLC method
- ○ Change in the sample solution led into positive improvement of the method performance but also decreased stability of samples to 1 day (due to lower pH)
- ○ Negative effect of all changes was rapid increase of LODs & LOQs, which can be dealt with by injection volume optimization
- ➢ Now we are focusing on the finalization of the method modifications and its basic validation
- ➢ Final steps will be the validation with our published SPE and real samples

ACKNOWLEDGEMENTS

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Diversity of Wheat in Accumulating Essential Metal Ions for Organic Farming: A Comprehensive Study of Micronutrient Enrichment

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INTRODUCTION

A significant part of the world's population suffers from micronutrient deficiencies. During the period of increasing grain production, the nutritional quality of wheat grain rather suffered, because the content of micronutrients in modern varieties is significantly lower compared to grain in landraces used a century ago. The enrichment of cereal grains with micronutrients such as zinc is, therefore, a priority area of research and requires a combination of knowledge and practices from the fields of agrotechnical, genetics, and plant physiology. By analyzing phenotypic, metabolic, and genetic diversity, we compare the ability of selected wheat varieties to accumulate specific micronutrients in tissues as well as the mechanisms contributing to the observed variability.

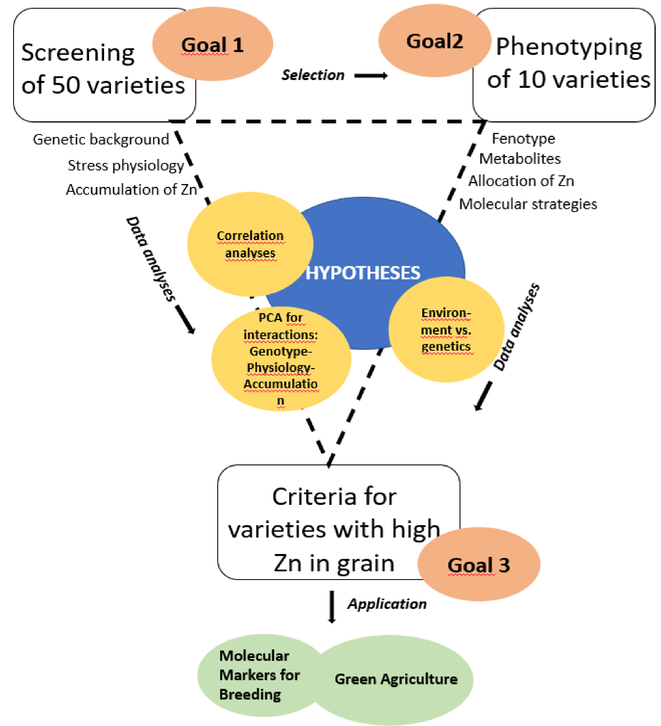
MATERIALS AND METHODS

Methodology of the project includes conventional laboratory procedures as well as phenotyping and sophisticated techniques of modern molecular methods. In addition, the project will implement detections of metal elements using positron emission tomography and direct gamma spectrometry for studying the uptake and distribution of toxic elements in the plant tissues.

AIM

The aim of the project is the evaluation and characterization of wheat genetic resources for micronutrient (Zn) uptake and accumulation. We also aim to clarify the ongoing molecular mechanisms, and design criteria for variety selection for organic breeding and agriculture.

PROJECT PLAN



RESULTS

Our hypothesis is that the integration of parameters such as the genetic background, defense mechanisms against (a)biotic stresses (including Zn toxicity), and the mechanisms for uptake (safe accumulation) of metals allow for the detection of of patterns (criteria) that can more reliably select varieties with a high content of micronutrients in grains. At the same time, some of the parameters might indicate to unique phenotypic plasticity of individual varieties, which is the result of ecophysiological adaptations to biotic stress (e.g. the activity of pathogenesis-related proteins like glucanases or chitinases). We will also verify the assumption that some of the 16 phenolic acids with a wide range of polarity, which we can analyze simultaneously in tissues of different durum wheat varieties, have an antioxidant and/or transport function and likely contribute to biofortification. According to the accumulation patterns of other antioxidants (e.g. enzymes) and/or indicators of metal chelation (content of folic acid) we expect to assign their probable function and value in terms of the bioavailability of accumulated microelements in wheat varieties. The illustrative figures show the preliminary results on variability of wheat varieties to Zn in growth media by means of growth restriction and proline accumulation.

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DETERMINATION OF PARA-ANISIDINE BY HPLC METHOD USING AZO COUPLING REACTION

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INTRODUCTION

Para-anisidine (PA) or 4-Methoxyaniline used as an insect repellent and ovicide. It is also used as and their derivatives are also widely used in chemical reaction as intermediates to obtain target materials such as pharmaceuticals, dyes, perfumes, photoinitiators etc. PA is a colorless crystals that easily oxidize and darken in air, odor is fishy.

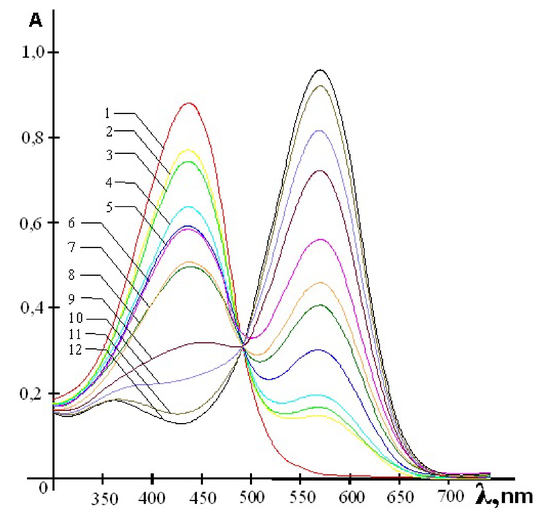
MATERIALS AND METHODS

Chromatography was performed on The Waters chromatograph with photo-diode array detector 996 on a Nova Pak column C_{18} (150×2.1 mm) was also used. The azo compounds separation was down with isocratic mode of mobile phase content (acetonitrile-water = 2:1). The mobile phase flow rate was 1 mL/min.

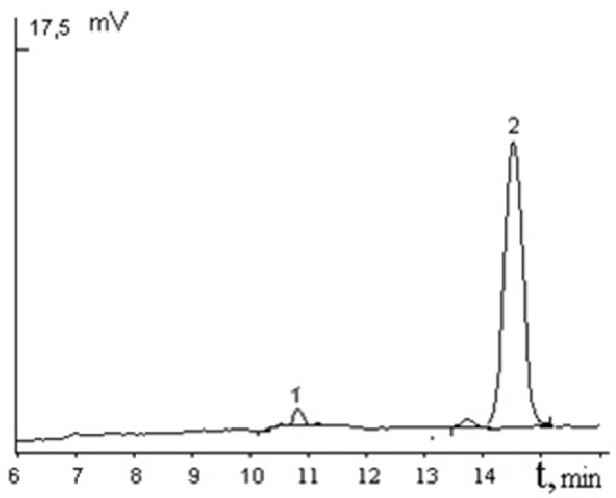
AIM

The goal is to use the azo coupling reaction to develop a highly sensitive method for determining residual amounts of p-anisidine in various objects by high-performance liquid chromatography.

RESULTS



The effect of solution pH on absorbance spectra of azo derivative of p-anisidine in 20% ethanol,
1-pH 6.45, 2-pH 11.3, 3-pH 11.45, 4-pH 11.75, 5-pH 11.9, 6-pH 12.0, 7-pH 12.25, 8-pH 12.3, 9-pH 12.8, 10-pH 13.2, 11-pH 13.5, 12-pH 13.6



Chromatograms of of azo derivative of p-anisidine

CONCLUSIONS

To determine p-anisidine, we used the azo coupling reaction with 4-nitrophenyl diazonium in the form of tetraphenyl borate salt.
The derivatization reaction was carried out in an aqueous-alcohol solution.
A method for determining PA in wastewater and soils has been developed.
Statistical processing of the results was carried out.

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