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DETERMINATION OF HEAVY METAL CONTENT (CD AND PB) IN CITRUS FEED RAW MATERIAL

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ABSTRACT

Heavy metal contamination of the food chain is a severe environmental problem that poses potential risks to human and animal health. In this regard, the safety of feed and feed materials is essential. In the present study, the levels of cadmium (Cd) and lead (Pb) in an innovative feed raw material – citrus waste (citrus pulp) by orange (*Citrus sinensis*), lemon (*Citrus limon*), red grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), lime (*Citrus aurantifolia*) and pomelo (*Citrus maxima*) were determined. The analysis was done by Atomic Absorption Spectrophotometer. The obtained results were compared with other literature data and with the requirements laid down in European and national legislation. Our results showed at the concentrations of cadmium and lead do not exceed the maximum permissible limits, which makes this citrus pulp safe for use as feed material in terms of Cd and Pb contamination.

Key words: heavy metals, cadmium, lead, citrus waste/citrus pulp, feed.

Introduction

Heavy metals

Globally, environmental pollution with heavy metals represents a serious ecological problem that poses a risk to human and animal health (Rajaganapathy et al. 2011; Makridis at al. 2012). One of the main sources of heavy metals for humans and animals is primary and secondary biological production, due to their property to accumulate along the food chain and especially in plant species (Duah 2012; Raja et al. 2016). In this regard, determining the levels of non-essential elements in feed raw materials is important for animal health, but also for the safety of food of animal origin (Dai et al. 2016; Adamse et al. 2017; Elliott et al. 2017). Heavy metal contamination of feed also leads to the bioaccumulation of toxic substances in animal manure, and from there in the soil and in the plant (Shi et al. 2011; Zhang et al. 2012, Zhou et al. 2020).

Cadmium and lead are highly toxic heavy metals and potentially hazardous to humans (Dehelean and Magdas 2013; Stancheva et al., 2014; Bataa et al. 2022). Moreover, they have a strong carcinogenic effect (Trichopoulos et al. 1997; Turkdogan et al. 2002) Cadmium accumulation leads to serious kidney and liver damage (Kara et al. 2004). Long-term exposures to cadmium can also lead to cardiovascular abnormality and osteotoxicity (Sharma et al.2015)

Lead is a cumulative toxicant affecting various organs and systems, with adolescents being particularly sensitive (WHO 2022). Lead poisoning seriously affects the renal, reproductive, and nervous system (Wani et al. 2015).

Waste feeding

In recent years, the disposal of organic waste and its use as feedstock has shown increasing interest (Alnaimy 2017). Citrus cultivation represents a major share of world fruit production (Luzardo et al. 2021). On the other hand, citrus juice production generates tons of waste products with excellent nutritional qualities that can be used as animal feed (Kasapidou et al. 2015; Andrianou et al. 2023). All this inevitably imposes the safety of this feed raw material in terms of animal and

human health. Regulation (EC) 183/2005 defines the requirements for feed hygiene and their safety. In addition, the European Commission determines the maximum permissible values for the content of cadmium and lead, regulated in Directive 2002/32/EC, amended by Regulation (EU) 1275/2013.

The aim of this study is to determine the concentration of cadmium and lead in the citrus waste by orange (*Citrus sinensis*), lemon (*Citrus limon*), red grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), lime (*Citrus aurantifolia*) and pomelo (*Citrus maxima*).

Materials and methods

The research material was obtained after the cold-pressed juice production of citrus fruits (by Industrial Freshly Squeezed Citrus Juice Machine (Luzzysa Exzel) and consisted of fresh citrus mash, peels, and seeds. The fruits used belong to the genus *Citrus L*. in the family *Rutaceae* and are: Orange (*Citrus sinensis*), Lemon (*Citrus limon*), Red grapefruit (*Citrus paradisi*), Mandarin (*Citrus reticulata*), Lime (*Citrus aurantifolia*), Pomelo (*Citrus maxima).* The final laboratory samples of all 6 species of citrus by-products were obtained after preliminary reduction of the aggregate sample composed of incremental samples which were taken from each batch according to the requirements of Regulation (EC) 152/2009 and are as follows:

Orange – aggregate sample composed of 28 incremental samples taken from 38 ton batch of waste product

Lemon – aggregate sample composed of 19 incremental samples taken from 18 ton batch of waste product

Red grapefruit – aggregate sample composed of 7 incremental samples taken from 2 ton batch of waste product

Mandarin – aggregate sample composed of 18 incremental samples taken from 16 ton batch of waste product

Lime – aggregate sample composed of 16 incremental samples taken from 12 ton batch of waste product

Pomelo – aggregate sample composed of 7 incremental samples taken from 1 ton batch of waste product

The production factory of freshly squeezed fruit and vegetable juices is located in a village Musachevo, Western Bulgaria. All samples were collected using clean triers and were placed in poly-lined leak resistant plastic bags. The plastic bags were sealed identified and transported to the laboratory in a cooler bag.

In the laboratory, the samples were weighed on a Sartorius analytical balance with an accuracy of 0.0001 g. Then it was placed in quartz crucibles and burned in an Ursamar RK 44 muffle furnace at 450^{o}C. After burning, the samples were mineralized with 6M HCL obtained from Hydrochloric acid fuming K 43922117 242 by Merck KGaA, Darmstadt, Germany. All total samples were analyzed for cadmium (Cd) and lead (Pb) concentrations. The analyses were done by Atomic Absorption Spectrophotometer (Perkin Elmer 5000). The method of analysis followed standard validated and internally developed validated methods for each chemical element. The level of Cadmium and Lead was measured at 283.30 and 228.8 nm wavelength, respectively.

Statistical Analysis

The summarized results of this study were presented as mean values (X) (mg/kg) fresh weight ± standard deviation (SD). The data were subjected to a statistical analysis with Student's-test to estimate the significance of values ($p < 0.05$).

Results and discussion

The use of citrus waste products as feed material is increasingly used in the nutrition of farm animals (Wadhwa et al. 2015; Luzardo et al. 2021). Many studies have been carried out regarding the chemical composition of this by-product and its valuable nutritional properties have been proven Fernandez-Lopez et al. 2004; Alnaimy 2017). But literature data on the content of heavy metals in citrus pulp and by – products are more limited. Most of the studies on heavy metal contents are in whole fruits or only in citrus peels. Citrus waste products and especially the peel have a high water content of about 80%, with the remaining dry matter containing pectin and polysaccharides, which increases their ability to accumulate toxic substances such as heavy metals (Husoon et al. 2013). According to some authors, the consumption of whole citrus fruits together with peels may have a beneficial effect on human health, although citrus peels have the potential to accumulate Cd and lead in larger amounts Czech et al. 2021.

The risk assessment of heavy metal contamination needs to be controlled even before they enter the food chain, through the observance of strict measures regarding the quality and safety of feed materials and strict compliance with the legislation (Elliott et al. 2017).

The obtained concentration of Cd and Pb citrus waste by orange (*Citrus sinensis*), lemon (*Citrus limon*), red grapefruit (*Citrus paradisi*), mandarin (*Citrus reticulata*), lime (*Citrus aurantifolia*) and pomelo (*Citrus maxima*) are presented in Table 1.

Table 1: Concentration of Cadmium (Cd) and Lead (Pb) in citrus waste.

Sample	Orange	Lemon	Red grape-fruit	Mandarin	Lime	Pomelo	Permissible limit (mg/kg)
Cd (mg/kg)	**< 0.05***	**< 0.05***	**< 0.05***	**< 0.05***	**< 0.05***	**< 0.05***	**1**
Pb (mg/kg)	**< 0.1***	**< 0.1***	**< 0.1***	**< 0.1***	**< 0.1***	**< 0.1***	**10**

**Method detection limits*

The results of this study indicate that the mean concentrations of Cd and Pb in citrus pulp samples were below detection limits. In the European and national legislation on maximum permissible levels of harmful substances (Regulation 1275/2013 and Ordinance 10/2009) norms for heavy metals in plant feed raw materials are set, as follows 1 mg/kg for cadmium and 10 mg/kg for lead. From the data in table 1 it is clear that the levels of cadmium and lead were below EU limits.

Different studies have investigated the concentrations of heavy metals in citrus pulp and peels. The results from a recent study for Cd and Pb content in citrus pulp and peels are presented on the table 2 (Czech et al. 2021). The data showed similar to our results, namely low levels of cadmium and lead in all investigated pulp and peel samples from orange, lemon, red grapefruit, mandarin, lime, and pomelo.

Table 2: Content of heavy metals (mg/kg) in citrus pulp and peel (Czech et al. 2021)

Sample	Part	Orange	Lemon	Red grapefruit	Mandarin	Lime	Pomelo
Cd(X±SD)	**Pulp**	**0.00015**	**0.00022**	**0.00015**	**0.00017**	**0.0191**	**0.00039**
Cd(X±SD)	**Peel**	**0.00049**	**0.00047**	**0.00191**	**0.00062**	**0.0265**	**0.00136**
Pb(X±SD)	**Pulp**	**0.01**	**0.0167**	**0.0247**	**0.00354**	**0.00004**	**0.0167**
Pb(X±SD)	**Peel**	**0.0102**	**0.0188**	**0.0301**	**0.0202**	**0.00046**	**0.0296**

**The original data were in µg/100 g*

Other authors studied the content of some heavy metals in citrus pulp (Ozcan et al. 2012). In their study, lead levels in grapefruit, mandarin, and lemon pulp were 0.26 mg/kg, 0.21 mg/kg, and 0.21 mg/kg, respectively. Although the cited data are higher than those presented in table 1, they again do not exceed the legal lead limit of 10 mg/kg for plant feed materials.

Other study showed analogous to our data for Cd and Pb concentration in orange pulp, as for both chemical elements the results are below the limits of quantification (Brima and Mohamed 2021).

Conclusion

The heavy metal levels in the citrus by – products we studied were compared with other reported literature data. Despite the different origins of samples, our results showed values below EU limits for plant feed materials. Based on this, it is concluded that citrus pulp obtained after the production of cold-pressed juice could be used as feed material without posing a risk to animal health in terms of cadmium and lead content.

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References

1. Adamse P., IneVan der Fels-Klerx H. J. and de Jong J. (2017). *Cadmium, lead, mercury and arsenic in animal feed and feed materials – trend analysis of monitoring results.* Food Additives & Contam-inants: Part A, 34:8, 1298–1311, doi: 10.1080/19440049.2017.1300686.
2. Alnaimy, A. (2017). *Using of Citrus By-Products in Farm Animals Feeding*. Open Access J. Sci., 1, 58–67. https://doi.org/10.15406/oajs.2017.01.00014.
3. Andrianou C., Passadis K., Malamis D. Moustakas K., Mai S., Barampouti E.M. (2023). *Upcycled Animal Feed: Sustainable Solution to Orange Peels Waste. Sustainability*. 15, 2033. https://doi.org/10.3390/su15032033.
4. Bataa B., Motohira K., Dugar D., Sainnokhoi T.A., Gendenpil L., Sainnokhoi T., Pelden B., Yohannes Y.B., Ganzorig S., Nakayama S.M.M. (2022). *Accumulation of Metals in the Environment and Grazing Livestock near A Mongolian Mining Area*. Toxics 2022, 10, 773. https://doi.org/10.3390/toxics10120773.
5. Brima E., Mohamed H. (2021). *Distribution of minerals between orange juice and orange flesh in various cultivars*. Czech J. Food Sci.. 2021;39(6):419–425. doi: 10.17221/258/2020-CJFS.
6. Commission Regulation (EC) No 152/2009 of 27 January 2009 *laying down the methods of sampling and analysis for the official control of feed*.
7. Commission Regulation (EU) No 1275/2013 of 6 December 2013 amending *Annex I to Directive 2002/32/EC of the European Parliament and of the Council as regards maximum levels for arsenic, cadmium, lead, nitrites, volatile mustard oil and harmful botanical impurities Text with EEA relevance*.
8. Czech A., Malik A., Sosnowska B., Domaradzki P. (2021). *Bioactive Substances, Heavy Metals, and Antioxidant Activity in Whole Fruit, Peel, and Pulp of Citrus Fruits*. Int J Food Sci. 2021 Mar 16;2021:6662259. doi: 10.1155/2021/6662259. PMID: 33816610; PMCID: PMC7990557.

9. Dai S.Y., Jones B.L., Lee K., Li W., Post L.O. and Herrman T. (2016). *Heavy Metal Contamination of Animal Feed in Texas*. Journal of Regulatory Science Vol. 4 No. 1
10. Dehelean A., Magdas D.A. (2013). *Analysis of mineral and heavy metal content of some commercial fruit juices by inductively coupled plasma mass spectrometry*. ScientificWorldJournal. Dec 18;2013:215423. doi: 10.1155/2013/215423. PMID: 24453811; PMCID: PMC3881688.
11. Directive 2002/32/EC of the European Parliament and of the *Council on undesirable substances in animal feed*.
12. Duah S. (2012). Thesis. *Determining the Level of Heavy Metal in Orange (Citrus sinensis) within Anglo Gold Ashanti, Obuasi Mine*.
13. Elliott S., Frio A. and Jarman T. (2017). *Heavy metal contamination of animal feedstuffs – a new survey*. Journal of Applied Animal Nutrition, 5, E8. doi:10.1017/jan.2017.7.
14. Fernandez-Lopez J., Fernandez-Gines J., Aleson-Carbonell L., Sendra E., Sayas-Barbera E., Perez-Alvarez J.A. (2004). *Application of functional citrus by-products to meat products*. Trends Food Sci. Technol. 2004, 15, 176–185.
15. Husoon Z.A., Al-Azzawi M.N.A., Al-Hiyaly S.A.K. (2013). *Investigation Biosorption Potential of Copper and Lead from Industrial WasteWater Using Orange and Lemon Peels*. Al-Nahrain Journal of Science, 16(2), 173–179, 2013. doi:10.22401/JNUS/16.2.27.
16. Kara H., Colakoglu N., Kukner A., Ozan E. (2004). *Structural changes of cadmium chloride that occurs in tissue of rat and effects of Metallothionein on these changes light: Microscobic work*. J Med Sci, 24, 6.
17. Kasapidou E., Sossidou E., Mitlianga P. (2015). *Fruit and vegetable Co-products as functional feed ingredients in farm animal nutrition for improved product quality*. Agriculture 2015, 5, 1020–1034. https://doi.org/10.3390/agriculture5041020.
18. Luzardo S., Banchero G., Ferrari V., Ibanez F., Roig G., Aznarez V., Clariget J. and La Manna A.(2021). *Effect of Fresh Citrus Pulp Supplementation on Animal Performance and Meat Quality of Feed lot Steer*s. Animals 2021, 11, 3338.https://doi.org/10.3390/ani11123338.
19. Makridis C., Svarnas C., Rigas N., Gougoulias N., Roka L., Leontopoulos S. (2012). *Transfer of heavy metalcontaminants from animal feed to animal products*. Journal of Agricultural Science and Technology A 2(1A):149–154.
20. Ordinance No. 10 of 04/03/2009 on the *maximum permissible concentrations of undesirable substances and products in the feed*.
21. Ozcan M.M., Harmankaya M., Gezgin S. (2012). *Mineral and heavy metal contents of the outer and inner tissues of commonly used fruits*. Environ Monit Assess. 2012 Jan: 184(1):313–20. doi: 10.1007/s10661-011-1969-y. Epub 2011 Mar 16. PMID: 21409363.
22. Raja R. O., Sobhanardakani S., Cheraghi M. (2016). *Health risk assessment of citrus contaminated with heavy metals in Hamedan city, potential risk of Al and Cu*. Environ. Health Eng. Manag.; 3 (3):131–135.
23. Rajaganapathy V., Xavier F., Sreekumar D. and Mandal P.K. (2011). *Heavy Metal Contamination in Soil, Water and Fodder and their Presence in Livestock and Products: A Review*. Journal of Environmental Science and Technology, 4: 234–249. DOI: 10.3923/jest.2011.234.249.
24. Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 *laying down requirements for feed hygiene*.
25. Sharma H., Rawal N. and Mathew B. (2015). *The characteristics, toxicity and effects of cadmium*. Int J Nanosci Nanotechnol 3:1–9 ISSN 1735-7004

26. Shi J.C., Yu X.L., Zhang M.K., Lu S.G., Wu W.H., Wu J.J. and Xu J.M. (2011). *Potential risks of copper, zinc, and cadmium pollution due to pig manure application in a soil-rice system under intensive farming: A case study of Nanhu, China*. J. Environ. Qual., 40, 1695–1704.

27. Stancheva M., Makedonski L., Ivanova K. (2014). *Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast*. Bulgarian Chemical Communications. 46. 195–203.

28. Trichopoulos D. (1997). *Epidemiology of cancer*. In Cancer, Principles and Practice of Oncology pp. 231–258. Lippincott, Philadelphia.

29. Turkdogan M. K., Kilicel F., Kara K., Tuncer I. and Uygan I. (2002). *Heavy metals in soil, vegetables and fruits in the endemic upper gastrointestinal cancer region of Turkey*. Environmental toxicology and pharmacology, 13, 175–179.

30. Wadhwa M., Bakshi M. P. S., Makkar H. P. S. (2015). *Waste to Worth: Fruit Wastes and by-Products as Animal Feed.* CABI Rev. 1–26. https://doi.org/10.1079/PAVSNNR201510031.

31. Wani A.L., Ara A. and Usmani J.A. (2015). *Lead toxicity: A review*. Interdisciplinary Toxicology.; 8(2): 55–64. https://doi.org/10.1515/intox-2015-0009.

32. World Health Organization (2022), https://www.who.int/news-room/fact-sheets/detail/lead-poisoning-and-health.

33. Zhang F., Li Y., Yang M., Li W. (2012). *Content of heavy metals in animal feeds and manures from farms of different scales in northeast China*. Int J Environ Res Public Health. Aug;9(8):2658–68. doi: 10.3390/ijerph9082658. Epub 2012 Jul 27. PMID: 23066389; PMCID: PMC3447579.

34. Zhou C.R., Ma Q., Yu W.T., Xia Z.Q., Ning C.C., Yuan H.Y., Li S.L. (2020). *Accumulation of heavy metals in soil and maize after 17-year pig manure application in Northeast China*. Plant Soil Environ., 66: 65–72.