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CHEMICAL ANALYSIS OF BIOACCUMULATED HEAVY METALS IN FORMULATED MEDICINAL PRODUCED FROM NATURAL PRODUCTS FOR INDUSTRIALIZATION.

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ABSTRACT

Bioaccumulation is essentially the build-up of contaminants such as heavy metals or pesticides in living organisms. That is, bioaccumulated heavy metals are those metals essentially build up in living organisms which are very pronounced among the aquatic organisms, because they absorbed contaminants from water around them faster than their bodies are able to excrete, which was the basic justification for this investigation on some selected fruits, domestically consumed. Whereas heavy metals are any metallic elements that have a relatively high density and toxic or poisonous even at low concentration, which is an environmental issue that has become a global problem. The chemical analysis of bioaccumulated heavy metals in formulated natural medicinal from ginger, onion, garlic, alligator pepper and bitter kola, was evaluated using instrumental techniques. The formulated samples contain (5g ginger, 4g onion, 3g garlic,2g bitter kola and 1g alligator pepper) each, but subjected to different drying techniques to ascertain the most probable method. Where samples A = sundried, B = air-dried, and C = oven-dried. The results shown that, lead ranges between (A=0.16 - B=0.5ppm), while it ranges between (A=0.07 - B=0.13ppm) for cadmium. But differs with copper (C=0.05 to B=0.09ppm), whereas for zinc (A&C=0.10 - B=0.92ppm), and nickel (A&C=0.06 - B=0.07ppm) the same order was observed. Therefore, the bioaccumulated heavy metals observed was on the safer side for human consumption when compare to the WHO (0.001-3mg/L) human consumption thresholds, with sun-dried techniques preferably.

Keywords: Bioaccumulated, Density, Environmental, Heavy Metals, Poisonous, Toxic.

1.0 INTRODUCTION

Heavy metal pollution is an environmental issue that has become a global problem. The production and emission of heavy metals has increased along with increased industrial development. This has led to increasing concern over food safety due to soil polluted with anthropogenic heavy metals released from industry or agriculture, such as smelting industries, residues from metalliferous mines, pesticides, fertilizers, and municipal composts (Guala et al.,2010). Heavy metals are chemical elements that have a relatively high density, strong toxic effects and pose an environmental threat. Heavy metals are of considerable environmental concern due to their toxicity, many sources, non-biodegradable properties, and accumulative behaviours (Nica et al.,2012). The presence of heavy metals in foods poses serious health hazards, depending on their relative levels. The ability of plants to accumulate metals and possibly other contaminants varies with both the nature of the plant species and the nature of the metal contaminant. Cereals, in this case of Zea mays L (maize), are known to be good accumulators of contaminants (Orisakwe et al.,2012).

Agricultural soils in many parts of the world are slightly to moderately contaminated by heavy metal toxicity, such as cadmium (Cd), copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co), chromium (Cr), lead (Pb), and arsenic (As). This could be due to long term use of phosphate fertilizers, sewage sludge application, dust horn smelters, industrial waste and poor watering practices in agricultural lands (Wang et al.,2017). The primary response of plants is the generation of reactive oxygen species upon exposure to high levels of heavy metals. Various metals either generate reactive oxygen species directly through Haber-Weiss reactions or overproduce reactive oxygen species and the occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity (Miedico et al.,2016). The indirect mechanisms include their interactions with the antioxidant system, disrupting the electron transport chain or disturbing the metabolism of essential elements (Afrin et al.,2015). One of the most deleterious



effects induced by heavy metals exposure in plants is lipid peroxidation, which can directly cause biomembrane deterioration. Malondialdehyde, one of the decomposition products of polyunsaturated fatty acids of a membrane, is regarded as a reliable indicator of oxidative stress. Such toxic elements are considered soil pollutants due to their widespread occurrence, and their acute and chronic toxic effect on plants grown. Several studies on the effects of bioaccumulation in plants through uptake of heavy metals from soils at high concentrations have been carried out and indicate great health risks, taking into consideration food chain implications. Utilization of food crops contaminated with heavy metals is a major food chain route for human exposure, especially those under continuous cultivation. The cultivation of such plants in contaminated soil represents a potential risk since the vegetal tissues can accumulate heavy metals (Eqani., 2016). Heavy metals become toxic when they are not metabolized by the body and accumulate in soft tissues Chronic ingestion of toxic metals has undesirable impacts on humans and the associated harmful impacts become perceptible only after several years of exposure (Mahmood et al.,2010).

Food crops are one of the important parts of our diet, and they may contain a number of essential and toxic metals (Wodaje et al., 2014). Depending on growing media characteristics. Vegetables are the major source of human exposure to heavy metal and contribute about 90 % of the total metal intake, while the rest 10 % intake occurs through dermal contacts and inhalation of contaminated dust Food safety is a burning issue regarding human health in the recent decades because of the high demand for food. This scenario leads to stimulate researchers and scientists to work on health risk associated with consumption of heavy metals, pesticides, and toxin-contaminated food (Bhattacharyya et al.,2016).

Essential and nonessential elements are regularly added to our food chain through excessive use of agrochemicals, municipal wastewater, industrial effluents, and raw sewage for irrigation (Elzwayie et al.,2017). The Agency for Toxic Substances and Diseases Registry (ATSDR 2011) has classified heavy metals and metalloids such as As, Pb, and Cd found in the environment as 1, 2, and 7 on the basis of toxicity. Some elements like Cu, Cr, Fe, Mn, and Zn are essential for animals and human beings because they play an important role in different metabolic functions, enzymatic activities, sites for receptors, hormonal function, and protein transport at specific concentrations (Hubbs et al., 2005). Other elements like As, Cd, and Pb are nonessential and have no beneficial role in plants, animals, and humans and have no nutritional function, as they are highly toxic. It is necessary to characterize the sources and contents of heavy metals in soil in order to establish quality standards and to determine the threats to human health and food safety (Hubbs et al., 2005). The associated health hazards of toxic metal depend on concentrations of these metals in specific media and exposure time. Long time and chronic exposure can cause health hazards even at low concentrations of toxic metal (Khan et al., 2011). The main objective of this study was to determine the bioaccumulated heavy metals in some selected fruits samples.

2.0 MATERIALS AND METHODOLOGY

2.1 MATERIALS

2.1.1 Apparatus

The following Apparatus were used; Crucibles, Beakers, Oven, Muffle furnace, Heating mantle, Volumetric flasks, Pair of forceps Analytical balance, Desiccators

2.1.2 Reagents

The reagent used were; Distilled water, HNO₃, H₂SO₄, HCl

2.1.3 Sample Collection

The samples were obtained by market survey from Oje market in Ede, Osun State.

2.1.4 Sample Treatment

Each of the ingredients were sliced into smaller sizes. Each sliced sample was divided into three equal parts; The first part was sun-dried, the second part was Oven- dried, while the third part was air-dried. The dried samples were then grounded into smaller particles with the aid of a mortar and pestle and sieved to obtain homogeneous fine particles which was subjected to further analyses.

2.1.5 Sample Formulation

The powdered ingredients were measured as follows; 5g of ginger, 4g of onion, 3g of garlic, 2g of bitter kola and 1g of alligator pepper in three folds, the first one was sun dried(A), the second one oven dried(B), while the third one was air dried(C). Each of this samples were thoroughly mixed for homogeneous reaction.

2.2 METHODOLOGY

2.2.1 Bioaccumulated Heavy Metal Content Determination

Procedure: **5m**l of **10%** per chloric acid (**HCI0**₃), Trioxonitrate (V) (**HN0**₃) and **15m**l of Hydrofluoric acid (**HF**) were all added to the ash sample after weighed. The solution was stirred properly with glass rod/police rod and allowed to evaporate as it was heated at a temperature of 60° C for 12hrs. 4ml of Hydrochloric acid (HCl) was then

added to the cooled solution and warmed to dissolve the samples or any salt formed. Then filtered with glass wool (care was taken with glass wool, hand glove as well as test tube holder was used because it is carcinogenic). On cooling, the solution was then diluted to 50ml with deionized water. The solution was then introduced into AAS for the reading. The final results were obtained.

3.0 RESULTS AND DISCUSSION

3.1 Results

3.1.1 Bioaccumulated Heavy Metal Content

The result presented in Table 1, showed the bioaccumulated heavy metal composition with respected to dried techniques.

Samples	Pb	Cd	Cu	Zn	Ni	
	ррт	ррт	ppm	ppm	ppm	
А	0.16	0.07	0.07	0.10	0.06	
В	0.50	0.13	0.09	0.92	0.07	
С	0.35	0.08	0.05	0.10	0.06	

Table 1: Bioaccumulated heavy metal content in samples

Key: A=Sun-dried sample, B=Oven-dried sample, C=Air-dried sample





3.2 Discussion

The levels of Pb, Cd, Cu, Zn, and Ni in the samples and their descriptive statistical parameters were shown in Table 1. Lead ranged from 0.16 - 0.50 ppm, where the least content was observed for sun-dried (A=0.16ppm) and highest with oven-dried (B=0.50ppm) sample. Although, the lowest limit of the above range was sparingly higher than the value set for foods or food products by the most regulatory bodies. Because, a maximum permissible limit of

1mg/kg was prescribed by the FAO/WHO (2002) for food additives, while the threshold values (mg/kg) for cereal grains, leafy vegetables and fruiting vegetables are 0.1, 0.2 and 0.05 respectively, (FAO/WHO, 2012). Differently, a value of 0.03 mg/kg was prescribed by (WHO, 1999) for medicinal plants in their final dosage form. This slight different may not be unconnected with the agricultural practice, the plant and the soil properties (such as cancerous nature of soil) that mighty affected the uptake of the metal which was in accordance with the work of Kitata et al (2012).

While the Cd (ppm) ranged from 0.07- 0.13, which fall within the required limits. With the highest value observed for oven-dried and least with sun-dried.

Copper was observed to have the range (0.37-0.90ppm), which was also slightly above the threshold for woman consumption, as recommended by WHO. This disparity hold for the same reason advanced for lead, which was in accordance with the work of Diriigen, (2010).

However, there has been dramatic increase in the use of Zinc based fertilizers and addition of sludge to the soil under plants, so it is important to monitor the zinc levels in spices. In this study, zinc levels ranges (0.10-0.90ppm). The most interesting part of it was that, both sun and air-dried recorded the lowest value (010ppm) but highest with the oven-dried (0.90ppm).

The same pattern was observed for nickel, which ranges thus (0.06-0.07ppm), where both the sun and air-dried sample recorded same value bearably minima, but highest with oven-dried. That is, the determined values are within tolerable limits, which was in accordance with the work of Kitata (2012). The above observation can be inferred from figure 1 above, which shown clearly at glance the level and distribution of those metals with respect to different dried techniques.

4.0 CONCLUSION AND RECOMMENDATION

4.1 Conclusion

The levels of Pb, Cd, Cu, Zn, and Ni in the spectrum of the samples was successfully determined and the health risk associated with intake of these bioaccumulated heavy metals has been indirectly assessed, with the reference to the available standards in accordance with global ethical practices. The accumulation of heavy metals by the active ingrident followed the pattern sun-dried, air-dried and oven-dried, in decreasing order. Inferred that, sun-dried is most suitable techniques for bioaccumulated heavy metals investigation in most edible samples.

4.2 Recommendations

The following recommendations were made after the study:

- Further study should be carried out on effect and toxicity level of bioaccumulated heavy metals on food.
- Further research should be carried out on the pattern of distribution of these bioaccumulated heavy metals.

References

Afrin, M. Y. Mia, M. A. Ahsan, and A. Akbor, "Concentration of heavy metals in available fish species (bain, Mastacembelus armatus; taki, Channa punctatus and bele, Glossogobius giuris) in the Turag river, Bangladesh," Pakistan Journal of Scientific and Industrial Research Series B: Biological Sciences, vol. 58, no. 2, pp. 104–110, 2015.

Al-Fraihat A. H. 2009. Effect of different nitrogen and sulphur fertilizer levels on growth,yield and qualityof onion (Allium cepa L.). Jordan Journal of Agricultural Science, 5:155-166.BAMPIDISV.A.,NITAS D. 2013. Arsenic, Cadmium, Lead andMercury as Undesirable Substances in Animal Feeds. AnimalScience andBiotechnologies, 46: 17-22

Ali and E. Khan, "Trophic transfer, bioaccumulation and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/webs: concepts and implications for wildlife and human health," Human and Ecological Risk Assessment: An International Journal, 2018, in Press.

Ali and E. Khan, "What are heavy metals? Long-standing controversy over the scientific use of the term' heavy metals'-proposal of a comprehensive definition," Toxicological & Environmental Chemistry, vol. 100, no. 1, pp. 6–19, 2018.

Alloway, "Sources of heavy metals and metalloids in soils,": Trace Metals and Metalloids in Soils and their Bioavailability, pp. 11–50, Springer, Dordrecht, Netherlands, 2013.

Appenroth, "What are "heavy metals" in Plant Sciences?" Acta Physiologiae Plantarum, vol. 32, no. 4, pp. 615–619, 2010.

Arunakumara, B. C. Walpola, and M.-H. Yoon, "Current status of heavy metal contamination in Asia's rice lands," Reviews in Environmental Science and Bio/Technology, vol. 12, no. 4, pp. 355–377, 2013.

AWilk, E. Kalisińska, D. I. Kosik-Bogacka et al., "Cadmium, lead and mercury concentrations in pathologically altered human kidneys," Environmental Geochemistry and Health, vol. 39, no. 4, pp. 889–899, 2017.

Aycicek M, Kaplan O and Yaman M. Effect of cadmium on germination, seedling growth and metal contents of sunflower (Helianthus annus L.). Asian J Chem. 2008; 20: 2663–2672.

Bampidis V.A., Nistor E., Nitas D. 2013. Arsenic, Cadmium, Lead and Mercury as Undesirable Substances in Animal Feeds. Animal Science and Biotechnologies, 46: 17-22.

Barwick and W. Maher, "Biotransference and biomagnification of selenium copper, cadmium, zinc, arsenic and lead in a temperate seagrass ecosystem from Lake Macquarie Estuary, NSW, Australia," Marine Environmental Research, vol. 56, no. 4, pp. 471–502, 2003

Bhattacharyya P, Chakrabarti K, Chakraborty A, Tripathy S and Powell MA. Fractionation and bioavailability of Pb in municipal solid waste compost and Pb uptake by rice straw and grain under submerged condition in amended soil. Geosciences Journal. 2016; 12(1): 41– 45. DOI: https://doi.org/10.1007/s12303-008-0006-9

Decena, M. Arguilles, and L. Robel, "Assessing heavy metal contamination in surface sediments in an urban river in the Philippines," Polish Journal of Environmental Studies, vol. 27, no. 5, pp. 1983–1995, 2018.

DeForest, K. V. Brix, and W. J. Adams, "Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration," Aquatic Toxicology, vol. 84, no. 2, pp. 236–246, 2007.

Diriigen, "Accumulation of heavy metals in freshwater organisms: assessment of toxic interactions," Turkish Journal of Chemistry, vol. 25, no. 2, pp. 173–179, 2010.

Dissanayake and R. Chandrajith, "Phosphate mineral fertilizers, trace metals and human health," Journal of the National Science Foundation of Sri Lanka, vol. 37, no. 3, pp. 153–165, 2009.

Dissanayake and R. Chandrajith, "Phosphate mineral fertilizers, trace metals and human health," Journal of the National Science Foundation of Sri Lanka, vol. 37, no. 3, pp. 153–165, 2009.

Duffus, "'Heavy metals" a meaningless term? (IUPAC Technical Report)," Pure and Applied Chemistry, vol. 74, no. 5, pp. 793–807, 2002.

Elzwayie . A, H. A. Afan, M. F. Allawi, and A. El-Shafie, "Heavy metal monitoring, analysis and prediction in lakes and rivers: state of the art," Environmental Science and Pollution Research, vol. 24, no. 13, pp. 12104–12117, 2017.

Eqani, R. Khalid, N. Bostan et al., "Human lead (Pb) exposure via dust from different land use settings of Pakistan: a case study from two urban mountainous cities," Chemosphere, vol. 155, pp. 259–265, 2016.

Ercal, H. Gurer-Orhan, and N. Aykin-Burns, "Toxic metals and oxidative stress Part I: mechanisms involved in metal-induced oxidative damage," Current Topics in Medicinal Chemistry, vol. 1, no. 6, pp. 529–539, 2001

Guala SD, Vega FA and Covelo EF. The dynamics of heavy metals in plant-soil interactions. Ecological

Modelling. 2010; 221: 1148–1152. DOI: https://doi.org/10.1016/j.ecolmodel.2010.01.003

Hubbs-Tait L, Nation JR, Krebs NF and Bellinger DC. Neurotoxicants, micronutrients, and social environments individual and combined effects on children's development. Psy Sci in the Pub Int, Supp. 2005; 6(3): 57–121.

Ihedioha JN, Ujam OT, Nwuche CO, Ekere NR and Chime CC. Assessment of heavy metal contamination of rice grains (Oryza sativa) and soil from Ada field, Enugu, Nigeria: Estimating the human health risk. Hum and Ecol Risk Assess: An Int J. 2016; 22(8).

Islam, R. Proshad, and S. Ahmed, "Ecological risk of heavy metals in sediment of an urban river in Bangladesh," Human and Ecological Risk Assessment: An International Journal, vol. 24, no. 3, pp. 699–720, 2018.

Jaishankar M, Tseten T, Anbalagan N, Mathew BB and Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology. 2014; 7(2): 60–72. DOI:

Järup, "Hazards of heavy metal contamination," British Medical Bulletin, vol. 68, no. 1, pp.167–182, 2003.

Kalafova, A., Kovacik, J., Capacarova, M., Kolesarova, A., Lukac. N., Stawarz, R., Formicki, G., Laciak, T. 2012.
Accumulation of zinc, nickel, lead and cadmium in some organs of rabbits after dietary nickel and zinc inclusion. Journal of Environmental Science and Health, Part A, 47: 1234-1238.

Khan S, Cao Q, Zheng YM, Huang YZ and Zhu YG. Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. Env Pollution. 2011; 152: 686–692. DOI:

Kitata R.H., Chandravanshi B.S. 2012. Concentration levels of major and trace metals in onion (Allium cepa L.) and irrigation water around Meki town and lake Ziway, Ethiopia. Bull. Chem. Soc. Ethiop., 26: 27-42

Longhurst R (2010). Global Leadership for Nutrition: The UN's Standing Committee on Nutrition (SCN) and its Contributions. Available at:http://opendocs.ids.ac.uk/opendocs/handle/123456789/5387Khan S, Shanz M, Jehan

N, Rehman S, Shah MT, Din I (2013).Drinking water quality and human health risk in Charsadda district, Pakistan. Journal of Cleaner Production, 60:93-101

Mackay A.K, M. P. Taylor, N. C. Munksgaard, K. A. Hudson-Edwards, and L. Burn-Nunes, "Identification of environmental lead sources and pathways in a mining and smelting town: mount Isa, Australia," Environmental Pollution, vol. 180, pp. 304–311, 2013.

Mahmood A. D. and R. N. Malik, "Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in Lahore, Pakistan," Arabian Journal of Chemistry, vol. 7, no. 1, pp. 91–99, 2014.

Markiewicz-Górka I., Januszewska L., Michalak A., Prokopowicz A., Januszewska E., Pawlas Jomova K., Valko M. 2011. Advances in metal-induced oxidative stress and human disease. Toxicology, 283: 65-87.

Merian E, Anke M, Inhat M and Stoeppler M. Elements and their compounds in the environment Wiley VCH, Weinhem, Germany; 2004. DOI: 10.1002/9783527619634

Miedico, A. Ferrara, M. Tarallo, C. Pompa, D. Bisceglia, and A. E. Chiaravalle, "Hazardous and essential trace elements profile in the different soft tissues of Lithophaga lithophaga (Linnaeus, 1758) from Southern Adriatic Sea (Italy)," Toxicological & Environmental Chemistry, vol. 98, no. 8, pp. 877–885, 2016.

Morgan A.J "Calcium-lead interactions involving earthworms: an hypothesis," Chemistry and Ecology, vol. 2, no. 3, pp. 251–261, 1986.

Mortvedt, "Heavy metal contaminants in inorganic and organic fertilizers," Fertilizer Research, vol. 43, no. 1–3, pp. 55–61, 1996.

Nagajyoti, K. D. Lee, and T. V. M. Sreekanth, "Heavy metals, occurrence and toxicity for plants: a review," Environmental Chemistry Letters, vol. 8, no. 3, pp. 199–216, 2010.

Ngo, S. Gerstmann, and H. Frank, "Subchronic effects of environment-like cadmium levels on the bivalve Anodonta anatina (Linnaeus 1758): III. Effects on carbonic anhydrase activity in relation to calcium metabolism," Toxicological & Environmental Chemistry, vol. 93, no. 9, pp. 1815–1825, 2011.

Nica, M. Bura, I. Gergen, M. Harmanescu, and D.-M. Bordean, "Bioaccumulative and conchological assessment of heavy metal transfer in a soil-plant-snail food chain," Chemistry Central Journal, vol. 6, no. 1, p. 55, 2012.

Nkansah MA, Amoako CO (2010). Heavy metal content of some common spices available in markets in the Kumasi metropolis of Ghana. American Journal of Scientific and Industrial Research 1(2):158-163.

Orisakwe, J. K. Nduka, C. N. Amadi, D. O. Dike, and O. Bede, "Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria," Chemistry Central Journal, vol. 6, no. 1, p. 77, 2012

Pujar, K.G.; Hiremath, S.C.; Pujar, A.S.; Pujeri, U.S.; Yadawe, M.S. Analysis of Physico-ChemicalandHeavy Metal Concentration in Soil of Bijapur Taluka, Karnataka. Sci.Revs. Chem. Commu. 2012, 2 (1), 76–79

S.-x. Liang, N. Gao, Z.-c. Li, S.-g. Shen, and J. Li, "Investigation of correlativity between heavy metals

concentration in indigenous plants and combined pollution soils," Chemistry and Ecology, vol. 32, no. 9, pp. 872–883, 2016.

Sharma N, Balaji PM, Deepika B, Swati DW (2014). Analysis of heavy metals content in spices collected from local market of Mumbai by using atomic absorption spectrometer. Food Chemistry 3(5):56-57.

Slaveykova and G. Cheloni, "Preface: special issue on environmental toxicology of trace metals," Environments, vol. 5, no. 12, p. 138, 2018.

Stasions S., Nasopoulou C., Tsikrika C., Zabetakis I. 2014. The bioaccumulation and physiological effects of heavy metals in carrots, onions, and potatoes and dietary implications for Cr and Ni: awreview. Journal of Food Science, 79: 765-780

Tangahu B.V., Abdullah S.R.S., Basri M.I., Anuar N., Mukhlisin, M. 2011. A review on heavy metals (As, Pb and Hg) uptake by plants through phytoremediation. International Journal of Chemical Engineering, Volume 2011, 31 pages

United Nations Environmental Protection. Global Program of Action; 2004. [Google Scholar]

United States Department of Health and Human Services, Agency for Toxic Substances and Disease Registry. Toxicological Profile for Cadmium; 2008

Wang F, Wang Z, Kou C, Ma Z and Zhao D. Responses of wheat yield, macro- and micronutrients, and heavy metals in soil and wheat following the application of manure compost on the North China plain. PLoS ONE. 2016; 11(1): e0146453 DOI:

Wang, W. Wu, F. Liu, R. Liao, and Y. Hu, "Accumulation of heavy metals in soil-crop systems: a review for wheat and corn," Environmental Science and Pollution Research, vol. 24, no. 18, pp. 15209–15225, 2017.

Wodaje, A.; Alemayehu, A. Levels of Essential and Non-essential Metals in Garlic (Allium sativum L.)Samples Collected from Different Locations of East Gojjam Zone, Ethiopia.JoMCCT, 2014, 5 (3), 10–19.

Zhao, X. Shi, C. Li, H. Zhang, and Y. Wu, "Seasonal variation of heavy metals in sediment of Lake Ulansuhai,

China," Chemistry and Ecology, vol. 30, no. 1, pp. 1–14, 2014 Zukowska J and Biziuk M. Methodological evaluation of method for dietary heavy metal intake. J Food Sci. 2008; 73(2): R21–R29. DOI: 10.1111/j.1750-3841.2007.00648.x