JJBS

Volume 11, Number 1,March 2018
ISSN 1995-6673
Pages 113 - 116

Jordan Journal of Biological Sciences

Short Communication

Arsenic Exposure from Bean Seeds Consumed in Owerri Municipal, Imo State, Nigeria: Can Insect Pest Detoxify the Metalloid during Infestation?

Luke C. Nwosu[1*], Usman Zakka[1], Beauty O. China[1] and Gerald M. Ugagu[2]

[1]Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State,
[2]Department of Science Laboratory Technology, Imo State Polytechnic Owerri, Nigeria

Received May 31, 2017; Revised September 17, 2017; Accepted September 21, 2017

Abstract

The common bean, *Phaseolus vulgaris* (L.) is one of the most important sources of protein worldwide. Bean consumption has increased in Nigeria particularly due to exorbitant cost of rice in the country. The present study determines the concentrations of total arsenic in samples of bean seeds obtained from the streets of Owerri city, Imo State, Nigeria using standard protocols. Bean seeds, sold in enclosed and open containers, are considered. The estimated dietary intake of total arsenic is compared with the arsenic benchmark dose lower confidence limit. Concentrations of total arsenic differed significantly across sampling locations. The pooled mean total arsenic was 0.47 µg/g while the estimated daily mean dietary intake of total arsenic was 32.9 µg/g for a typical adult in the place. This value is below the permissible limit of 18-480 µg/day for an adult at the average weight of 60 kg established by the European Food Safety Authority. This suggests that bean seeds, sold and consumed in Owerri Municipal, do not have inherent and acquired arsenic-associated health risks. Bean seeds, exposed to the bean bruchid, *Acanthoscelides obtectus* Say (Col., Bruchidae) for 60 days in the laboratory at a temperature of 29.7^{o}C and RH of 71.7%, revealed that the insect pest showed a potential for detoxification using feeding as a mechanism. Evaluation of the potentials of *A. obtectus* in the detoxification of bean seeds contaminated with arsenic under long storage duration is suggested.

Keywords: *Phaseolus vulgaris*, Safety evaluation, Arsenic, Health risks, *Acanthoscelides obtectus*, Detoxification.

1. Introduction

The common bean, *Phaseolus vulgaris* (L.), is commonly consumed by humans worldwide and is one of the main sources of protein in developing countries (Lopes *et al*., 2016). The common bean is the most important pulse in human diet (Larochelle *et al*., 2016), and pulses are critical in achieving sustainable goals on food security, health and poverty alleviation. The exorbitant price of rice in Nigerian markets (167 % increases in cost over a period of two years) has led to an increase in bean consumption. Moreover, the global public advocacy on the health benefits of plant-based proteins favor bean consumption (Larochelle *et al*., 2016). It is simple to predict that the increased bean consumption will cause an upsurge in its production. Increases in bean consumption and production justify the need for safety assessment and risk management, ensuring that levels of environmental contaminants are below the maximum limits established by regulations or concerned authorities.

One of the contaminants of potential concern is arsenic, which can be released from anthropogenic sources, such as manufacturing of pesticides, smelting of metals, the use of arsenic-containing wood preservatives and production of pharmaceuticals (Adeyemi *et al*., 2017). Arsenic can also be released during glass production. Mining, metal smelting and burning of fossil fuels are the major industrial processes that contribute to arsenic contamination of the environment (soil, air and water). Apart from the man-made sources of environmental arsenic, there are natural sources. The earth's crust is an abundant natural source of arsenic and it occurs in at least 200 minerals, arsenopyrite, being the most common (FHE, 2017). According to Adeyemi *et al*. (2017), arsenic is the 20th most abundant element in the earth crust, its mean concentration in igneous and sedimentary rocks 2 mg/kg. Natural sources of arsenic may be found in inorganic and organic forms (FHE, 2017). The inorganic type is of geological origin and is found in ground water. Bangladesh, India and Taiwan have high concentrations. Organic arsenic is mainly found in sea-dwelling organisms; terrestrial species are not totally excluded. The source notwithstanding, arsenic is very toxic to human life (Adeyemi *et al*., 2017). It can be genotoxic and cytotoxic since it can induce micronuclei deoxyribonucleic acid strand breaks, chromosomes aberration and cell membrane distortion (Faita *et al*., 2013). Indeed, arsenic contamination is of great concern to human and environmental health. If ingested, symptoms of arsenic poisoning include diarrhea, vomiting, vomiting blood, blood in the urine, cramping muscles, stomach pain and

* Corresponding author. e-mail: luke2007ambition@yahoo.com.

convulsions. Most times, the human skin, kidneys and liver are affected (Mazumder and Dasgupta, 2011). Considering the increase in human activities that potentially release arsenic to the environment, there is a need to carryout safety assessment for this toxic metalloid element. Most previous studies on dietary intake of arsenic used total arsenic in making inference (Roychowdhury *et al*., 2003; Marti-Cid *et al*., 2008). This has been confirmed in a recent study by Adeyemi *et al*. (2017). Therefore, the first two objectives of the present study are to determine the total arsenic levels in bean from different locations in Owerri city, Imo State, Nigeria and to estimate its daily dietary intake through bean consumption.

In literature, it has been recorded that postharvest insects cause nutrient loss during feeding (Lale, 2002; Nwosu, 2016). Unfortunately, the potential uses of insects in the detoxification of metalloids, such as arsenic, are still largely untested. Given that insect-association with stored beans is inevitable, it is worth investigating if insects can help to detoxify the toxic elements contaminating bean seeds. Thus, as a third objective, the present study sought to examine the potential of insects using the bean bruchid, *Acanthoscelides obtectus* Say (Col., Bruchidae) in the detoxification of arsenic. *A. obtectus* is one of the most important storage pests in Africa (Giga *et al*., 1992). Dry weight losses during storage of bean seeds are between 10-40% but where management is poor losses can be above 50% (Paul, 2007). This scientific communication on the detoxification of arsenic by *A. obtectus* could provide new insights in the use of insects in detoxification of toxic elements.

2. Materials and Methods

2.1. Sample Collection

Two bean samples were taken from markets/stores in each of the five prominent streets (n = 10) in Owerri Municipal, Imo State (5^o 28’ 35.6” (5.4766^o) N; 7^o 1’ 0.6” (7.0168^o) E. The streets sampled were Douglas, Whetheral, Rotibi, Okigwe Road and World Bank area. These streets (Douglas and Whetheral in particular) are exclusive points where commercial activities hold due to concentration of business outfits, such as markets, banks, mini shops and offices. During sample collection, bean sold in enclosed (sample coded a) and open containers (sample coded b) were considered. Samples were transferred immediately into clean plastic cork-containers and labeled also immediately according to point of collection. Sample digestion and determination of total arsenic were achieved the next day.

2.2. Determination of Total Arsenic

Total arsenic in each bean sample was determined using the method of Nardi *et al*. (2009). We added 1 mL 20 % (v/v) HNO_3 and 2 mL 30 % (v/v) H_2O_2 to 50 mg of the milled (electric mill) and sieved (0.4 mm screen) bean samples. The mixtures were digested for 40 min in a microwave oven. The digests were allowed to cool and their volumes brought up to 15 mL with distilled water. Arsenic was determined using an atomic absorption spectrometer (Varian Spectr AA 220FS). The accuracy and precision of the results were verified using four replications from each of the ten samples. Quality control using certified reference material (containing 1000 µg/ mL arsenic) was achieved through arsenic re-run as guided by Adeyemi *et al*. (2017).

2.3. Estimation of Daily Intake of Arsenic through Bean Consumption

The daily intake of arsenic was estimated using the formula explained in Adeyemi *et al*. (2017). The Estimated Daily Intake (EDI) in mg/day/individual or µg/day/individual = Cm X Mg, where Cm is the average concentration of element in a cereal sample (ng/g or µg/g) and Mg is the mass of bean consumed daily.

The quantity of bean consumed daily by a typical Nigerian adult (average weight 60 kg) was assumed to be 70 g which is the quantity of rice consumed daily by a typical Nigerian adult (Africa Rice Center, 2005).

2.4. Safety Evaluation

The estimated daily intake of arsenic was compared to the benchmark dose lower confidence limit value for arsenic. The standard is 0.3-8.0 µg arsenic per kg body weight/day. This is equivalent to 18-480 µg/day for an adult at the average weight of 60 kg (EFSA, 2009; Adeyemi *et al*., 2017). If the observed mean value falls within the range, it implied there is no arsenic health risks associated with bean consumption. On the contrary, if the observed mean value exceeds the range, it implied there is arsenic health risks associated with bean consumption.

2.5. Insect Culture

The bean bruchid, *A. obtectus* adults were isolated from naturally infested bean in Port Harcourt, Rivers State, Nigeria and authenticated using morphological characteristics (Lale, 2002). A total of 400 unsexed adults were identified and used to start the culture. Two plastic containers (height 28 cm; diameter 18 cm) with 300 g of landrace bean seeds were used and each received 200 beetles. Each container was covered immediately with muslin net (held in place with cut container-lid) for protection and ventilation. The parent beetles were allowed to feed on the culture seeds and lay eggs for a period of 8 days and thereafter, they were sieved out of the containers. The two set-ups were kept for 4 weeks for new adults to emerge under ambient temperature and RH of 29.7^{o}C and 71.7%, respectively. Two-week old emerged adults were used for the detoxification assay.

2.6. Insect Detoxification Assay

The experiments were conducted in the Crop Protection Laboratory of the Department of Crop and Soil Science, University of Port Harcourt, Rivers State, Nigeria. The mean ambient temperature and relative humidity of the laboratory were 29.7^{o}C and 71.7%, respectively. Fifty grammes of each of the 10 bean samples were weighed into ten different containers. Ten unsexed *A. obtectus* adults were introduced into each container and covered immediately with muslin net (held in place with cut container-lid) for protection and ventilation. These were arranged in a completely randomized design on laboratory bench. A control (without insects) of four replicates was designated. The bruchids were allowed to feed on the samples for 60 days. Post-infestation arsenic was determined using the above methods. Pre- and post-infestation arsenic levels were compared statistically to ascertain the occurrence and extent of detoxification caused by the insect. Comparable weights of infested and un-infested bean samples were used to accommodate the weight loss in infested samples. The experiments were repeated four times.

2.7. Statistical Analysis

The assumption for homogeneity of group variance was tested prior to analysis of variance using Levene's test (Somta *et al*., 2008) for equality of variances. The outcome of Levene's test eliminated the need for data transformation. Data on total arsenic levels in the bean samples were statistically analyzed using one-way analysis of variance. Upon significance of the F-test, means were separated (at posthoc test) using Honestly Studentized range (HSD). The student-t test was used to compare the difference between pre- and post-infestation arsenic levels. Statistical inference was based on α level of 0.05 and the p-values were flagged by the analytical software, SPSS (Statistical Software for the Social Sciences) version 21.0 (Nwosu *et al*., 2017).

3. Results and Discussion

Table 1 presents the results for total arsenic concentrations in the bean samples. Total arsenic differed significantly ($P < 0.05$) among the samples collected at different locations in major commercial streets in Owerri Municipal, Imo State, Nigeria. The location-specific mean values of total arsenic ranged from < 0.001 to 1.08 µg/g. These values are much lower than those reported for rice samples from South West Nigeria (50.37-78 ng/g) (Adeyemi *et al*., 2017), India (70-80 µg/kg) (Williams *et al*., 2005), Brazil and China (223-360 µg/kg) (Meharg *et al*., 2008; Batista *et al*., 2011). The pooled mean total arsenic resulting from the study (n = 10) is 0.47 µg/g. This is also lower than the total arsenic (2.88 µg/g) in pooled samples (n = 10) in oysters collected from local market in Port Harcourt, Nigeria (Amayo *et al*., 2016). Geographical variation in total arsenic is consistent with the literature (Adeyemi *et al*., 2017) and summarily related to differences in environmental quality (soil, air, water and intensity of the anthropogenic sources). Spatial variation in the level of total arsenic recorded in the present study may be attributed to differences in the soils on which the beans were cultivated. This is because the grains of cereals planted in arsenic-rich soils appear to have higher concentration of arsenic (Sahoo and Mukherjee, 2014). Anthropogenic sources of arsenic are location-specific and probably, bean samples exposed and sold in locations with more sources of arsenic are potentially more vulnerable to environmental contamination. Therefore, it is not surprising that bean samples collected from the streets of Douglas and Whetheral had higher concentrations of total arsenic. Briefly, there are sources of arsenic pollution near the two streets that could lead to higher arsenic concentrations. As the centre of commercial activities with high human population and activities, the environmental quality of soil, air and water (which is obviously lower than the other streets) probably account for the higher arsenic concentrations observed. This analysis is supported by literature (Amayo *et al*., 2016; Adeyemi *et al*., 2017).

Reports of human exposure from the ingestion of food contaminated with arsenic are considerably documented (Roychowdhury *et al*., 2003). Exposure to toxic arsenic is inevitably associated with health problems for humans. At chronic exposure, human lungs, buccal cavity, pharynx and other important organs face increased chances of cancer (Adeyemi *et al*., 2017). These malicious effects of arsenic can be preventable through routine testing for contamination. The results of the present study show that the estimated daily dietary intake of total arsenic through bean consumption by typical adults in the area is 32.9 µg. This reveals that the total arsenic intake linked to the consumption of beans in major commercial streets in Owerri Municipal, Imo State, Nigeria does not constitute threat to the health of the residents.

Table 1. Levels of total arsenic in bean samples (n = 10) sold in major commercial streets in Owerri Municipal, Imo State, Nigeria during March 2017

Sample codes	Streets sampled	Total arsenic (µg/g)
1a and 1b	Douglas	1.08 ± 0.01^{a}
2a and 2b	Whetheral	0.76 ± 0.05^{a}
3a and 3b	Rotibi	0.02 ± 0.03^{b}
4a and 4b	Okigwe road	$<0.001 \pm 0.00^{c}$
5a and 5b	World Bank	$<0.001 \pm 0.00^{c}$

Data are means ± standard error of the means of four replications. Means in a column followed by the same letter are not significantly different (α = 0.05) by HSD.
Pooled mean = 0.47 µg/g (used subsequently to compute the estimated daily intake of arsenic).
a = bean samples sold in enclosed container.
b = bean samples sold in open container.

After a two-month storage duration, some of the bean samples infested with *A. obtectus* lost some concentration of total arsenic (Table 2). However, loss of total arsenic due to insect feeding was not significant ($P > 0.05$). The highest percent detoxification that occurred in the study is 14.18 and on the contrary, some samples did not experience change in total arsenic level even when the insect fed for 60 days. Generally, it appears the beetles had an effect but only when arsenic values were higher (at Douglas and Whetheral) (Table 2). The other three sites had low arsenic values (≤ 0.02), that the beetles could not make much difference. From the observations above, there may be a threshold level of toxin that must be present for there to be any discernible effect of detoxification by the beetles. However, the result shows that the test insect can be potentially employed in achieving detoxification of bean contaminated with arsenic. At short exposure period, insect feeding was beneficial in reducing arsenic contamination especially added the fact that the test variety of bean was not broken down. Thus, there is a need to extend the storage duration beyond 60 days to observe what happens at prolonged storage conditions. Having the beetles feed longer may not make a difference at sites with low arsenic values but might make a difference on beans containing high arsenic concentrations (the present study provided the hint). Under the influence of insecticides and storage containers commonly used to protect stored bean seeds against beetle attacks in Nigeria, seeds can be successfully preserved for one year without being broken down. So, extension of the storage duration to examine the extent of detoxification may not be such a wasteful idea, the present study concludes.

Table 2. Pre- and post-infestation total arsenic and extent of detoxification caused by the bruchid insect, *Acanthoscelides obtectus* Say

Sample Codes	Streets Sampled	Pre-infestation total arsenic (μg/g)	Post-infestation total arsenic (μg/g)	Total arsenic lost (μg/g)	Percent Detoxification
1a and 1b	Douglas	1.08 ± 0.01^{a}	0.92 ± 0.01^{a}	0.16 ± 0.01^{a}	14.81 ± 0.01^{a}
2a and 2b	Whetheral	0.76 ± 0.05^{a}	0.70 ± 0.05^{a}	0.06 ± 0.05^{b}	7.89 ± 0.05^{b}
3a and 3b	Rotibi	0.02 ± 0.03^{b}	0.02 ± 0.03^{b}	0.00 ± 0.03^{b}	0.00 ± 0.03^{c}
4a and 4b	Okigwe road	$<0.001\pm0.00^{c}$	$<0.001\pm0.00^{c}$	$<0.001\pm0.00^{b}$	0.00 ± 0.00^{c}
5a and 5b	World Bank	$<0.001\pm0.00^{c}$	$<0.001\pm0.00^{c}$	$<0.001\pm0.00^{b}$	0.00 ± 0.00^{c}

t-values were not significant at $P > 0.05$.
Data are means ± standard error of the means of four replications. Means in a column followed by the same letter are not significantly different ($\alpha = 0.05$) by HSD.

4. Conclusion

In conclusion, beans consumed in the main commercial streets of Owerri Municipal at the time of the present study are safe for human health in regard to arsenic level. Total arsenic concentrations varied significantly among the locations sampled. Intra-spatial variation was linked to differences in the number and intensity of anthropogenic sources of the toxic element in these commercial streets. Variation in the concentration of arsenic (arsenic in soils where the bean was grown) may not be excluded. The present study found that the total arsenic contamination varies from place to place and this provides the rationale for location-specific analysis. The geographical variation is strongly related to environmental quality of soil, air, water and human activities. The present study has provided an important hint in the use of insects in the detoxification of toxic elements contaminating stored products. Further studies are suggested at long time storage duration.

References

Adeyemi JA, Adedire CO, Martins-Junior AC, Paulelli AC, Awopetu AF, Segura FR, Oliveira-Souza VC, Batista BL and Barbosa Jr F. 2017. Arsenic speciation in rice consumed in south-western Nigeria, and estimation of dietary intake of arsenic species through rice consumption. *Toxicol Environ Chem*, pages 1 - 8, DOI: 10.1080/02772248.2016.1269331.

Africa Rice Center (WARDA). 2005. Report of rice consumption in Nigeria.

Amayo KO, Horsfall Jr. M and Feldmann J. 2016. Analysis of arsenic in oysters using reversed phase HPLC-ICP-MS/ESI-ORBITRAP-MS. *Ife J Sci*, **18 (3)**: 703 - 709.

Batista BL, Souza JMO, de Souza SS and Barbosa Jr. F. 2011. Speciation of arsenic in rice and estimation of daily intake of different arsenic species by Brazilians through rice consumption. *J Hazardous Mater*, **191**: 342 - 348.

EFSA (European Food Safety Authority). 2009. EPSA Panel on contaminants in the food chain: scientific opinion on arsenic in food. *EFSA J*, **7 (10)**: 1351.doi: 10.2903/j.efsa.2009.1351.

Faita F, Cori L, Bianchi F. and Andreassi MG. 2013. Arsenic-induced genotoxicity and genetic susceptibility to arsenic-related pathologies. *Inter J Environ Res Pub Health* **10**: 1527 - 1546.

FHE (Facts on Health and the Environment). 2017. http://www.greefacts.org/en/arsenic/1-2/arsenic-2.htm (accessed May 24, 2017).

Giga DP, Ampofo JKO, Nahdy S, Negasi F, Nahimana M and Msolla SN. 1992. On-farm storage losses due to bean bruchids and farmers' control strategies. Occasional publications 8 CIAT, Cali, Colombia.

Lale NES. 2002. **Stored-Product Entomology and Acarology in Tropical Africa.** Mole Publications Ltd., Maiduguri, Nigeria.

Larochelle C, Katungi E and Cheng Z. 2016. Household consumption and demand for bean in Uganda: determinants and implications for nutrition security. Invited paper presented at the 5th International Conference of the African Association of Agricultural Economists, September 23 - 26, 2016, Addis Ababa, Ethiopia.

Lopes LM, Araújo AEF, Santos ACV, Santos VB, Sousa AH. 2016. Population development of *Zabrotes subfasciatus* (Coleoptera: Chrysomelidae) in landrace bean varieties occurring in Southwestern Amazonia. *J Econ Entomol*, **109 (1)**: 467 - 471.

Marti-Cid R, Ilobet JM, Castell V and Domingo JL. 2008. Dietary intake of Arsenic, Cadmium, Mercury and Lead by the population of Catalonia, Spain. *Bio Trace Element* Res, **125**: 120 - 132.

Mazumder DG and Dasgupta UB. 2011. Chronic arsenic toxicity: study in West Bengal, India. *Kaohsiung J Med Sci*, **27**: 360 - 370.

Meharg AA, Lombi E, Williams PN, Scheckel KG, Feldmann J, Raab A, Zhu Y and Islam R. 2008. Speciation and localization of arsenic in white and brown rice grains. *Environ Sci Technol*, **42**: 1051 - 1057.

Nardi EP, Evangelista FS, Tormen L, Saint Pierre TD, Curtius AJ, de Souza SS and Barbosa F. Jr. 2009. The use of inductively coupled plasma mass spectrometry (ICP-MS) for the determination of toxic and essential elements in different types of food samples. *Food Chem*, **112**: 727 - 732.

Nwosu LC. 2016. Chemical bases for maize grain resistance to infestation and damage by the maize weevil, *Sitophilus zeamais* Motschulsky. *J Stored Prod Res*, **69**: 41 - 50.

Nwosu LC, Adedire CO, Ogunwolu EO and Ashamo MO. 2017. Toxicological and histopathological effects of *Dennettia tripetala* seed used as grain protectant, food, and medicine. *Food Quality and Safety*, pages 1 - 9, DOI:10.1093/fqsafe/fyx019.

Paul UV. 2007. Bean pest management in East Africa - a scientific evaluation of organic insect control practices used by Tanzanian farmers. PhD dissertation, Federal Institute of Technology, Swiss.

Roychowdhury T, Tokunga H and Ando M. 2003. Survey of arsenic and other heavy metals in food composites and drinking water and estimation of dietary intake by the villagers from an Arsenic affected area of West Bengal, India. *Sci the Total Environ*, **308**: 15 - 35.

Sahoo PK and Mukherjee A. 2014. Arsenic fate and transport in the ground water-soil-plant system: an understanding of suitable rice paddy cultivation in arsenic enriched areas. In: Sengupta D (Eds.), **Recent Trends in Modeling of Environmental Contaminants**. Springer Publishers.

Somta C, Somta P, Tomooka N, Ooi PAC, Vaughan DA and Srinives P. 2008. Characterization of new sources of munbean (*Vigna radiate* (L.) Wilczek) resistance to bruchids, *Callosobruchus* spp. (Coleoptera: Bruchidae). *J Stored Prod Res*, **44**: 316 - 321.

Williams PN, Price AH, Raab A, Hossain SA, Feldmann J and Meharg AA. 2005. Variation in arsenic speciation and concentration in paddy rice related to dietary exposure. *Environ Sci Technol*, **39**: 5531 - 5540.