Vol. 12(1), pp. 17-23, January-June 2020 DOI:10.5897/JECE2019.0450 Article Number: C34107163047 ISSN: 2141-226X Copyright ©2020 Author(s) retain the copyright of this article http://www.academicjournals.org/JECE



Journal of Environmental Chemistry and Ecotoxicology

Full Length Research Paper

Ingestion of lead-contaminated vegetables could affect the intelligent quotient of school children

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Received 25 October, 2019; Accepted 12 February, 2020

Lead (Pb) is a potential environmental contaminant that has the capability of causing some human health problems, especially when it accumulates in food crops. This study aimed to evaluate the risk of ingesting Pb-contaminated vegetables on the intelligent quotient (IQ) of school children, using "target risk quotient" methodology. From the responses to the questionnaires administered to the school children/teachers, vegetables ingestion rate (V_{IR}) by the children was found to be 16.28±2.59 g/child/day while the average body weight of the 100 selected school children, aged 6 to 8 years was 32.5±2.8 kg. Total estimated daily intake (EDI) of Pb from contaminated vegetables was 3.45 mg/day/child while that of the control was 0.098 mg/day/child. The calculated target risk quotients were 0.985 and 0.028, for children in the lead-mining community and in the control group, respectively. The evaluated intelligent quotients (IQ) were respectively 92.35±13.23 and 106.95±11.75 for children from lead-mining community and the control. These values were not significantly different at p < 0.05 while the risk quotient was less than 1 (< 1). The overall result suggests that exposure of school children to lead-contaminated vegetables alone, at the concentrations established in this work, would not compromise their IQ.

Key words: Children, intelligent quotient, lead, vegetables.

INTRODUCTION

The accumulation of lead and zinc in soil, water and vegetation in Ishiagu and Uburu communities of Ebonyi State, Nigeria has been reported (Oje et al., 2010) and this could have several health implications for the people living in that environment. The Zamfara Pb poisoning episode in two communities in Nigeria, Bukuyou and Anka, was a very severe case of Pb toxicity (Anetor et al.,

2016). One of the immediate dangers inherent in illegal or artisanal mining is immediate death of the miners when a mining pit collapses (Okutu, 2013). Infants and young children are especially sensitive to even low levels of Pb, which are associated with behavioural problems, learning deficits, and lowered IQ (Zhang et al., 2012; Bellinger et al., 1992). Vulnerability of children to Pb toxicity was due

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> to the fact that they are at a stage of rapid brain development. It has been established (World Health Organization (WHO), 2010) that children and adults are exposed to Pb most probably through inhalation of contaminated dust particles or aerosols, drinking water and food. Some studies (McLaine et al., 2013; Mazumdar et al., 2011; Wasserman et al., 1997) have reported that some adverse impacts of Pb exposure on a child may include low cognition-test scores, memory loss, learning deficit, fine motor skills' impairment and behaviourial distortion.

Adverse effect of lead exposure on children

Lead is among the metals widely distributed in the environment and is suspected of causing developmental neurotoxicity. It has been demonstrated that, unlike other heavy metals in its group, Pb at very low concentration easily crosses the blood-brain barrier and the placenta (Wasserman et al., 1997), and can transfer 57.4% of the lead concentration to the fetus (Wang et al., 1989; Bellinger and Needleman, 2003). Based on its inherent toxicity, at a low concentration of <10 p.g/dl, it has a direct effect on the developing brain and hence on children's intellectual ability (Bellinger and Needleman, 2003; Lidsky and Schneider, 2003). The manifestations of the negative effect of Pb in children include irritability, behavioural changes, hyperactivity or decreased activity, that is, dullness, loss of developmental milestones and language delay. A recurrent theme in research on childhood Pb poisoning over the past 40 years has been its toxicity to the developing nervous system at levels previously thought to be safe (Needleman, 2009) but it was demonstrated (Baghurst et al., 1992) that low-level exposure to lead during early childhood was inversely associated with neuropsychological development through the first seven years of life. It was stated (Live et al., 2015) that intelligent quotient (IQ) is a score which is generally derived from a variety of tests to assess human intelligence. Such studies have always been of major interest in cognitive neuroscience. Although taking different tests for the same age or the same test at different occasions show varying scores, clinical psychologists in general adopt IQ score as a statistically valid metric for evaluating cognitive functions in children (Woodberry et al., 2008).

Several epidemiological studies have shown that lead exposure in early childhood causes a discernible IQ impairment (Tong et al., 1996; Pocock et al., 1994; Schwartz, 1994) which may be irreversible (Needleman et al., 1990). The potential for adverse effects of Pb exposure in children is heightened because their physiological uptake rates are higher than those in adults and since their systems are not fully developed, they would be undergoing rapid development and consequently more vulnerable than adults (World Health Organization (WHO), 1995).

Statement of the problem/aim of the study

Pb is highly toxic even at a low blood level concentration (10 p.g/ dl), hence brain development in children and their intellectual ability are adversely affected when ingested (Bellinger and Needleman, 2003; Lidsky and Schneider, 2003). Therefore evaluation of its effect on children will necessitate regular blood sampling. Apart from ethical issues, refusal by uninformed rural dwellers to willingly donate blood generates bottle neck in conducting the required tests. Hence an application which circumvents blood sampling (non-invasive method) would be a very welcome approach.

Since consumption of Pb-contaminated vegetables can take lead directly to the gastrointestinal tract, this work was carried out to evaluate the risk of ingesting Pbcontaminated vegetables on the school children's intelligent quotient (IQ).

MATERIALS AND METHODS

Scope and design of the study

In this work questionnaires were used to elicit some vital information that guided the study, such as estimating the pattern/daily intake of the vegetables by the children; obtaining the number of primary school children aged 6 to 8 years, who were born/schooling in the Pb-mining community; those whose parents have resided in the Pb-mining community for over 25 years and finally those residing at distances ≤ 0.5 km away from the mining pit.

For the sampling of lead contamination of the selected vegetables, two communities, one with high activities of lead-mining for over 25 years and another community 40 km away without any known mining activities (control), both in Ebonyi State, Nigeria were used.

Ethical consideration

Cultivation of vegetable crops in farms is a common practice in most communities in Nigeria and the village communities in Ebonyi State are not exception. Since the study adopts non invasive procedure of taking blood samples, ethic consent was not required. However permissions were obtained from the farm owners in the local community from where the vegetable samples were collected. The simple method or technique of cutting portions of the vegetables/crops was not considered stringent to affect endangered or protected species.

Study population and parameters

The population of the study comprised a total of two hundred and forty (240) children in the Pb-mining community and control (community 40 km away from the mining site). Parameters evaluated include the average body weight and age of each child, the estimated daily intake (EDI) of each vegetable by each child, concentrations of Pb in the 6 selected vegetable samples cultivated in the Pb-mining community and control and the IQ of each of the 100 selected school children.

Samples and collection

Vegetable crops cultivated in the two communities used in the

study, Vernonia amygdalina (bitter leaves), Pterocarpus mildbraedii (ora leaves), Ocimum gratissimum (scent leaves), Abelmoschus esculentus (okra pods), Murraya koenigii (curry leaves) and Solanum melongena (garden eggs), were harvested directly from the farms, with permission from their owners. The fresh vegetable crops were separately packaged in different sterile polythene bags and were labeled by their names as well as names of the respective sites where they were harvested.

Analysis of Pb in the vegetable samples

The vegetable samples were thoroughly washed with distilled water with 3 changes to remove dust and soil particles. The washed vegetable samples were dried in an oven at 50°C for 8 h as prescribed (Douglas and Wolfgang, 1995), and then milled using a blender. They were digested using the modified method (Allen et al., 1996).

Each of the prepared vegetable samples (3.0 g) was carefully put into digestion flasks containing 3 ml of 60% hydrochloric acid and 10 ml of 70% nitric acid. The mixture in each digestion flask (in triplicate for each sample) was then placed in a fume cupboard for digestion until clear solution was obtained. Each mixture was allowed to cool and then filtered through a Whitman's No 1 filter paper. Each was made up to 100 ml in a standard volumetric flask with de-ionized water.

The concentrations of Pb in each of the prepared vegetable samples were determined using AA-700 Shimadzu Model Atomic Adsorption Spectrophotometer. A blank for zeroing the instrument was prepared by adding a mixture of 3.0 ml of 60% hydrochloric acid and 10 ml of 70% nitric acid to 3g of de-ionized water in a digestion flask. Mean concentrations of Pb in the various vegetable samples were determined from the standard graph generated by the instrument and recorded in mg/kg.

Evaluation of the intelligent quotient of school children

A total of one hundred (100) school children comprising fifty two (52) and forty eight (48) children were respectively selected from the Pb-mining area and control. They were administered some cognitive function tests (Raven's Standard Progressive Matrices and psychometrics) and their scores converted to intelligence quotient (IQ) values accordingly (Angoff, 1984).

Risk assessment of lead (as contaminant) in vegetables

The human risk assessment of lead-contaminated vegetables intake by the children was calculated using target risk quotient (TRQ) (USEPA, 2010), as modified (Onuoha et al., 2016).

$$TRQ = \frac{EDI}{IR_{fDose}}$$
(1)

Where: Ingestion Reference Dose (IR_{fDose}) for lead is 3.5 x 10⁻³ mg/kg/day (Moslem and Miebaka, 2015).

The estimated daily intake (EDI) of Pb for each child was calculated using the modified equation below (Ugbomeh and Jaja, 2013):

$$EDI = \frac{\mathbf{E}_F \times \mathbf{E}_D \times \mathbf{V}_{IR} \times \mathbf{C}_M \times 10^{-3}}{\mathbf{W}_{AR} \times \mathbf{T}_A}$$
(2)

Where, E_F = Exposure frequency (365 days/year); E_D = Exposure duration (child: 6 years); V_{IR} = Vegetable ingestion rate (from

questionnaire analysis; g/child/day); C_M = Lead concentration in the vegetable (mg/kg); W_{AB} = Average body weight of the children (Culled from our questionnaire: 32.5 kg); T_A = Average exposure for non-carcinogens (equal to $E_F \times E_D$)

Since average exposure for non-carcinogens (T_A) equals (E_F x $E_D),$ the final equation becomes

$$EDI = \frac{\mathbf{V}_{IR} \times \mathbf{C}_{M} \times 10^{-3}}{\mathbf{W}_{AR}}$$
(3)

Therefore the final equation for target risk quotient (TRQ) is given:

$$TRQ = \frac{\mathbf{V}_{IR} \times \mathbf{C}_{M} \times 10^{-3}}{\mathbf{W}_{AB} \times \mathbf{IR}_{fDose}}$$
(4)

Statistical analysis

The data generated from intelligence quotient tests were analysed using SPSS version 21.0 and results expressed as mean \pm standard deviation of intelligence quotient (IQ) scores. Students't-tests for independent samples for the lead-mining area and control were used to compare the means. Pearson correlation test was used to assess association between total lead concentrations in the vegetable crops and IQs of the children. P-value < 0.05 was considered as significant (two-tailed analysis). Other results are expressed as mean \pm standard deviation and their significance analysed also at P-value < 0.05.

RESULTS

Questionnaire analysis

From a total of two hundred and forty (240) questionnaires distributed to the school children in the Pb-mining area and control, fifty two (52) and forty eight (48) children were selected from the Pb-mining community and control respectively for cognitive tests; they were aged 6 to 8 years. The above selection was based on the fact that their parents have lived in the two communities for \geq 25 years and these children were born and schooling within the community. The average body weight of the children selected was 32.5 ± 2.8 kg while the cumulative vegetable ingestion rate (V_{IR}) was 16.28 ± 2.59 mg/kg/child.

Mean concentrations of lead (mg/kg) in vegetable samples

The mean concentrations of lead in the 6 selected vegetables from the lead-mining community and control shown in Table 1 are quite different. For the Pb-mining community the values ranged from 0.46 ± 0.06 to 1.97 ± 0.05 mg/kg and that of control from 0.01 ± 0.00 to 0.06 ± 0.02 mg/kg. Based on the regular pattern of consumption of these vegetables (as deduced from the questionnaires) the total Pb consumed by each child were 7.24 ± 0.88 and 0.07 ± 0.03 mg/day respectively for lead-mining

Sample —	^a Mean concentrations of lead (mg/kg) in vegetable	
	Lead-mining community	Control
Vernonia amygdalina	0.56 ± 0.11	0.01 ± 0.00
Pterocarpus mildbraedii	1.97 ± 0.05	Not detected
Ocimum gratissimum	0.46 ± 0.06	Not detected
Abelmoschus esculentus	1.51 ± 0.22	0.05 ± 0.01
Murraya koenigii	0.81 ± 0.21	0.06 ± 0.02
Solanum melongena	0.93 ± 0.23	0.01 ± 0.00
Total conc. of lead in vegetables	6.24 ± 0.88	0.07 ± 0.03

Table 1. Mean concentrations of lead (mg/kg) in vegetables.

^aValues are mean of 3 analyses.

community and control. *Pterocarpus mildbraedii*, *Abelmoschus esculentus* and *Murraya koenigii*, have higher concentrations of lead when compared with other vegetables studied.

Estimated daily intake (EDI) of lead (mg/day) and target risk quotients (TRQs)

The estimated daily intake (EDI) of lead (mg/day) through ingestion of the six vegetable samples from lead-mining and control communities are shown in Table 2. The average body weight of children $(32.5 \pm 2.8 \text{ kg})$ obtained from the questionnaires was used in the calculations. The total estimated daily intake { Σ (EDI)_A} of lead by the children dwelling in the lead-mining community and control were 3.45 and 0.098 mg/day and the target risk quotients (TRQs) were 0.985 and 0.028 respectively.

Risk assessment and cognitive functions

The potential risk of consumption of lead-contaminated vegetables by school children was evaluated using target risk quotient (TRQ) (Equation 4). The value obtained for Pb-mining community was 0.985 (< 1) while for the control the value was 0.028 (< 1). The mean intelligent quotient (IQ) of 100 school children from lead-mining (52) and control (48) communities based on the results of cognitive functions tests were respectively 92.35±13.23 and 106.95±11.75 and these results were not significantly different (p<0.05).

DISCUSSION

Questionnaire administration

A questionnaire is a research tool primarily used to collect information from a population of individuals in a specific geographical area. The questionnaires administered in this work consisted of a series of questions and other prompts which enabled the identification of the focused population of school children in Ishiagu (Pb-mining community). They are those who were born and at the time of this work are attending a primary school in this community. They are also those whose parents have lived in Ishiagu for at least 25 years. Hence these selected children were considered to have been well exposed to Pb-contaminated vegetables, starting from their parents, their conception and birth, up till their current age (6-8 years), bearing in mind that Pb at very low concentration can easily cross the blood-brain barrier (9) and the placenta to affect the fetus (Nashashibi et al., 1999; Wang et al., 1989).

Lead in vegetables

Lead found in vegetables is basically derived from the environment in which it is grown. It can be seen from the results presented in this work that some vegetables grown in the same farm accumulated different levels of lead. For example *Pterocarpus mildbraedii* and Abelmoschus esculentus accumulated more lead that other crops and hence could be termed hyperaccumulator. Exposure or consumption of more of these hyper-accumulators may therefore contribute more to the risk of lead exposure. Agricultural crops grown near heavily travelled roads or industrial sources of lead can also have significant concentrations because of air borne lead deposited on them. The total mean concentration of lead in the vegetables cultivated in the lead-mining community was significantly higher than that in the reference control community. This strongly suggests that the high concentration of lead found in the vegetables harvested from the lead-mining community is largely derived from lead tailings or dust from the mining activities. Differences in concentrations of heavy metals in vegetables grown in urban and metal smelter contaminated sites have been reported (Kachenko and Singh, 2006). Also high concentrations of lead (10 μ g/m³) have been reported in some plants grown in close proximity to lead mining sites as opposed to the

Vegetable	Lead-mining community	Control
	EDI (mg/day)	EDI (mg/day)
V. amygdalina	0.280	0.005
P. mildbraedii	1.00	-
O. gratissimum	0.007	-
A. esculentus	0.560	0.026
M. koenigii	1.125	-
S. melongena	0.478	0.005
	∑(EDI) _A = 3.45	$\sum (EDI)_{C} = 0.098$
	*TRQ = 0.985	*TRQ _{Control} = 0.028

 Table 2. Estimated daily intake (EDI) of lead and calculated target risk quotients (TRQs).

* $\Sigma TRQ = (\Sigma EDI)/(IR_{fDose}).$

concentrations (7.6 x $10^{-5} \mu g/m^3$) for plants grown in far distances from mining sites (Beloian, 1985). This was reflected in the vegetables harvested from the lead-mining community and control that is 40 km away from the mining site. The very little amount of lead found in vegetables cultivated in control could be from other sources such as road traffics.

Risk evaluation

Risk assessment is a term used to describe the overall process or method where a particular chemical or toxicant may have the potential to cause harm or distortion with respect to a set end point or parameter. It examines the nature and degree of risk based on a set of mathematical equations relating estimated daily intake (EDI) and ingestion rate (IR) of the chemical (directly or indirectly). This relationship known as target risk quotient (TRQ) is the methodology in use to perform risk analysis of the potential effect or exposure of a substance such as lead and at the concentration at which no risk or adverse effects are expected. If the risk quotient is calculated to be less than 1 (< 1), then no adverse health effects are expected as a result of exposure but if the risk quotient is greater than 1 (>1), then adverse health effects are possible.

Considering the total mean concentrations of lead in the vegetables cultivated in the lead-mining community and that in control, it is obvious the difference was significant. This means that children in Pb-mining community stand the risk of more intake of lead from contaminated vegetables. This gave rise to higher estimated daily intake than in control. However this value was slightly below the Tolerable Daily Intake (TDI), even though lead intake from food can generally be variable (World Health Organization (WHO), 1993). In spite of this variability, instrumentation weaknesses and other intrinsic sources, the methodology adopted for this work is acceptable for risk analysis. It should also be noted that children from these communities can still be exposed to lead in different ways ranging from deteriorating lead paint in their homes, lead-contaminated dust and soil, Pb in water from leaded supply lines or plumbing, and through other crops. It has been estimated that 40 to 50% of dietary lead is absorbed in children, whereas in adults normally 5 to 10% of dietary lead is absorbed from the gastrointestinal tract (World Health Organization (WHO), 1993). Based on the fact that Pb in food are transported directly to the gastrointestinal tract, it is scientifically reasonable to evaluate the concentrations in the vegetables regularly consumed in large quantities as a determinant of exposure in school children living in the Pb-mining community or where active artisanal mining is taking place. A study with infants (Ryu et al., 1983) where a mean intake of 3-4 µg Pb/kg b.w. was not associated with an increase in blood lead concentration suggests that blood lead concentration may directly be related to the concentration in the food taken daily. Hence it is expected that the blood lead concentration in children consuming more of Pb-contaminated vegetables should be higher than their parallel control. Using Tolerable Daily Intake (TDI) of 3.6.mg/kg as standard reference (World Health Organization (WHO), 1995), the result of the total estimated daily intake (EDI) of lead (3.45 mg/day) obtained in this work suggests that the children in this community may not develop lead-related health problems or any negative challenges (EDI < TDI).

Target risk quotient (TRQ), derived from target hazard quotient is a complex parameter used for the estimation of potential (health) risks associated with long term exposure to chemical pollutants (34). Here, the interpretation of TRQ value is binary; that is, TRQ is either > 1 or < 1. Value of TRQ >1 indicates a reason for health concern (Bassey et al., 2014). Results presented in this work showed that the target risk quotients (TRQs) predicted for the consumption of the various vegetables was less than 1 (<) 1 for the children in both communities, although the value for the lead-mining community was significantly higher than for the control.

This implies that the consumption of the vegetables cultivated in lead-mining community may not result to any health concern such as impairment of IQ. This is supported by our result whereby TRQ is less than 1 (TRQ < 1). Also the mean IQ scores of the children tested in the two communities were not significantly different (p<0.05). This may suggest that within the threshold of the lead contamination of the vegetables, the likelihood of a negative effect of lead on the cognitive function of the exposed children is remote.

Conclusion

Uncontrolled lead-mining can bring about contamination of vegetables grown within the vicinity and consumption of such vegetables by children could present some risks, depending on the concentrations in the crops being cultivated and consumed in the community. Since all sources of possible exposure to the children, such as drinking water and other lead-contaminated food were not taken into consideration, risk assessment based only on the vegetables consumed by the children in the Pbmining community may not be adequate to give or provide accurate assessment of the risk associated with lead exposure, in terms of its effect on cognitive function of children who have been proven to be more prone to lead toxicity. This follows the fact from reports (Wang et al., 1989; Bellinger and Needleman, 2003) that lead at blood lead concentrations <10 p.g/dL has a direct adverse effect on the developing brain and children's intellectual ability. Not considering the socioeconomic status of the parents (education and occupation) could influence the results of this study (Kaufman, 2001). However, the result of this investigation could be important in working out strategies for ameliorating the risk of lead exposure through contaminated vegetables or The approach of not taking blood of other crops. children, as shown in this work, will be handy in risk assessment of environmental lead exposure to cognitive function of children.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors sincerely appreciate all the teachers in the Primary schools in the Pb-mining community and control who were involved in one way or the other in making this work a reality, together with all the parents of the children for lending their support. They particularly thank Professor Ikechukwu Onwurah of the Department of Biochemistry, University of Nigeria, Nsukka, who is currently on Sabbatical at Department of Basic Medical Biochemistry, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria for assistance in the instrumentation and useful suggestions, especially in the analysis of Pb in the vegetables. No grant from funding agencies was obtained for this work.

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