Evaluation of the Concentrations of some heavy metals(Pb, Cd and Cr) and long term exposure due to daily Consumption of ready-to-eat foods sold at Petrol station's Atmospheric conditions (AF) in Calabar Metropolis.

Abstract – just add the place and duration of the study

Background: Heavy metals contamination has been reported at petrol station environments. There is a possibility of contamination foods around petrol stations.

Objectives: In this study, the concentrations of Pb, Cd and Cr, long term exposure and daily consumption of ready-to-eat food foods sold at Petrol station's Atmospheric conditions (AF) in Calabar Metropolis was evaluated.

Methods: Foods samples, including such prepared ready-to-eat foods were collected at the point of sale at the fuel stations, about 7:00am in the morning before they were opened for sale (and exposed to the environment of the filling stations). These were labelled "Before". At about 2:00pm to 3:00pm same day, the same ready-to-eat food samples were collected again at the same spots.

Results: In this study, the levels of Pb, Cd and Cr were determined in some ready-to-eat foods that are sold around the filling station environments in Calabar metropolis. The results obtained, as presented in Figures 1-3, showed that the levels of Pb and Cd were significantly ($p<0.05$) increased in garri, afang soup, melon soup, white rice, beans, stew and meat pie, while the level of and Cr was significantly ($p<0.05$) increased in afang soup, melon soup, white rice, beans, stew and meat pie after 6 hours of exposure to petrol station's atmospheric conditions.

Conclusion: From this study, it may be concluded that exposure of ready-to-eat foods at the filling station's atmospheric conditions may cause heavy metal contamination to the foods, particularly Pb, Cd and Cr.

Key words: lead, Cadmium, Chromium, contamination and petrol stations

Introduction – References to be sequentially numbered and put in square bracket like [1]

Ready-to-eat foods are those foods that are considered to be ready to consume instantly at the point of sale. Clarence *et al*. (2009) and Mahakarnchanakul *et al*. (2010) reported that these foods may be consumed in either raw or cooked form, hot or cold as well as consumed with or without further heat treatment. As reported by Tambekar *et al*. (2008) ready-to-eat foods on the street are relatively cheap and readily accessible, accounting for the commonly available feeding source for many city dwellers. Hence, street foods play important roles in the feeding pattern of different categories of people in major urban cities in developing countries. It has been reported that about 80% of Thai ready-to-eat foods are generally eaten uncooked and are exported to USA and Europe (Jocelyn and Naewbanji, 2005). The ready-to-eat foods sold by food vendors and hawkers in streets and other public places usually have high patronage probably due to convenience, nutritional quality and flavor of the food. The sale of these foods helps to provide the needed source of income for the non-skilled people in such cities, thereby contributing to economic development of countries where such activities take place.

According to the FAO/WHO (2007), street foods may be obtained from a street side vendor, often from a makeshift stall which could also be portable. Generally, certain appealing factors that make street foods popular as food sources in most cities in the developing countries include familiarity, organoleptic property of the food, low cost and convenience in getting the food (Mahakarnchanakul *et al*., 2010). There exists a social pattern characterized by increased mobility, large number of itinerary workers and less family or home centered activities in developing societies where these foods are sold. This situation, has led to an increase to the increase in the amount of ready-to-eat foods taken outside the home. This therefore has led to the proliferation of food vendor services with the responsibility of good manufacturing practices of food being "transferred from individuals/families to the food vendors who usually do not enforce such practices" (Musa and Akande 2002; Clarence *et al*., 2009). In Nigeria, storage of these food products is done under poor hygienic conditions. More often than not, the products are displayed in open trays in open market places as well as hawked along the street by hawkers. Contamination of food may occur at any point in the production chain (i.e. from the point of harvest and transport of the raw materials, processing of the raw products, packaging, transportation of finished product, storage and marketing) to the final point of consumption. Due to poor processing methods used, these foods could therefore be contaminated by micro-organism, heavy metals and pesticides. Data regarding metallic concentration of food products at the point of consumption is necessary to allow for estimation of human exposure to these metals (Iwegbue, 2011).

Basically, Governments and many government agencies globally are known to have initiated several attempts to improve food safety, but food borne illnesses arising from food contamination is reported to still possess a significant health threat to humans in both developed and developing countries (Gasaluck, 2012). The ready-to-eat foods sold along streets are considered to be of risk to public health as a result of the difficulty associated with the control of quality of large number of street food vending operations. This difficulty is reported to be attributed to the diversity of the food, food mobility and the temporary nature of the process as well as inadequate basic infrastructure and services (Ghosh *et al*., 2007; DeSausa, 2008). Foods may be contaminated by the introduction of finely dispersed particles in the atmosphere into foods. Entry of these finely dispersed particles into foods may occur mainly due to the preparation and pre/post processing method used, wear and tear of metallic cooking vessels as well as environmental pollutants.

The levels of metal content in foods is of great importance because of the huge role that metallic ions play in health and disease (Hague *et al*., 2008). The human body cannot tolerate certain metals (like Cadmium and lead) at even low concentrations due to the fact that they are highly toxic (Suppin *et al*., 2006). Heavy metals may cause toxic responses by displacing a physiologically appropriate metal. For example, "cadmium can replace copper and iron in cytoplasmic and membrane proteins, with the free metal ions promoting the generation of free radicals (superoxide and hydroxyl radical) which in turn can lead to oxidative damage of lipids, nucleic acids and proteins" (Marias & Blackhurst, 2009). Cadmium has been implicated in the development of skeletal damage (Jarap, 2003). Cadmium and lead have been reported to harm reproductive system and embryonic development.

The physiologic roles of essential metals have been well documented. For example, Iron (plays the role as a haemopoietics of hemoglobin and cytochromes) (Marias & Blackhurst 2009). The physiologic roles of essential metals are due to the fact that these metals are constituents of proteins. A deficiency of these elements could induce disease conditions. For example, a deficiency of copper could induce elevated blood pressure, induce hypercholesterolemia and increase low density lipoprotein content in the blood which could trigger cardiac arrest. Similarly, a deficiency of manganese could cause chronic diseases like osteoporosis and diabetes mellitus. Intake of essential metals above threshold limits could cause toxicity problems. Epidemiological data have shown that there is a correlation between excessive dietary intake of zinc and an increased prevalence of obesity and other related diseases (Singh & Taneja, 2010).

As in Nigeria and many other countries, ready-to-eat foods account for a significantly large proportion of the daily food intake of individuals and families. A survey of revealed that only a handful of literature exists for the content of metals in Nigerian foods and these studies are limited in scope with respect to the type of element and food surveyed (Iwegbue, 2011). For example, Onianwa *et al*. (2001) examined the levels and daily intake of Cu and Zn from confectioneries (sweets, biscuits and breads). Currently, there is insufficient information regarding elemental composition of ready-to-eat foods consumed in southern Nigeria and no real study on individual and combined target hazard quotient values for common confectioneries has been reported, this study therefore seeks to provide a comprehensive evaluation of the concentrations, daily intake and long term exposure to metals due to consumption of ready-to-eat foods with a view to provide valuable information on the risks associated with their consumption.

Materials and Methods.

Materials

The following equipment and glass wares were used in the course of this research: Laboratory mortar (model EW-63100-60, from Cole-parmer company Ltd, USA), Evaporating plate (model SER-No.62, from Gallenkamp company Ltd, UK), Atomic Absorption Spectrophotometer (model AA6800, Schemadzu company, Japan).

Reagents/chemicals

Standard reagents and chemicals were used and include: Lichens coded International Atomic Energy Agency (IAEA-336), from Sigma, USA, Nitric acid (Riedel-deHaën, Germany), Perchloric acid (Sigma-Aldrich, Germany), Hydrofluoric acid, Ethanol, Methanol, Ethyl-acetate, (British Drug House Chemicals Ltd, Poole, England), distilled deionized water (obtained from Cross River State water board, Calabar-Nigeria).

Methods - References to be sequentially numbered from introduction and put in square bracket; mention statistical analysis followed

Collection of food samples

Foods samples, including such prepared ready-to-eat foods as garri, meat pie, stew, rice, beans, afang and melon soups were collected at the point of sale at the fuel stations, about 7:00am in the morning before they were opened for sale (and exposed to the environment of the filling stations). These were labelled “Before”. At about 2:00pm to 3:00pm same day, the same ready-to-eat food samples were collected again at the same spots (after they have been exposed to the filling stations atmospheric environment). These were labelled “After”. The food samples were collected in such a way that all the Garri purchased before, across the different filling stations were mashed together as a single sample, while the ones purchased after were equally collected to form a single sample. The other food samples were handled in in like manner.

Preparation of food samples for heavy metal analysis

One gram (1.0g) each of the **pulled** (means??)samples was weighed into a beaker. 20ml of aqua-rega (a solution of Nitric acid and perchloric acid (3:1) was added and the beaker was covered with glass for the initial effervescence to subside. Thereafter, the beaker was placed on a hot plate and heated to near dryness at about 80-90^{0}C. The aqua-rega was added as required in the course of digestion, to avoid drying. After the sample was fully digested, giving light coloured solution, the beaker was transferred onto a work bench and allowed to cool. The cooled sample was filtered into a 50ml beaker and made up to the mark with distilled water. This was transferred into a sample container in preparation for heavy metal (elemental) determination using Atomic Absorption Spectrophotometer (Awofolu, 2005).

Analytical Quality Assurance

To make sure that the analytical methods used for heavy metal determination are reliability, standard reference materials, Lichens coded (International Atomic Energy Agency; IAEA-336) were also digested and then analyzed using same procedure. Comparison of determined values with certified elemental values was carried out to ensure reliability of the analytical method used (Udiba *et al*, 2012).

RESULTS – to this only add discussion at relevant places; mention the values of metals present in ready to eat foods, even you can give to bars in the graph and also X-axis require type or name of the food sample

Results showing the different concentrations of Pb, Cd and Cr in some ready-to-eat meals before and after 6 hrs of exposure to petrol station’s atmospheric conditions (AF)

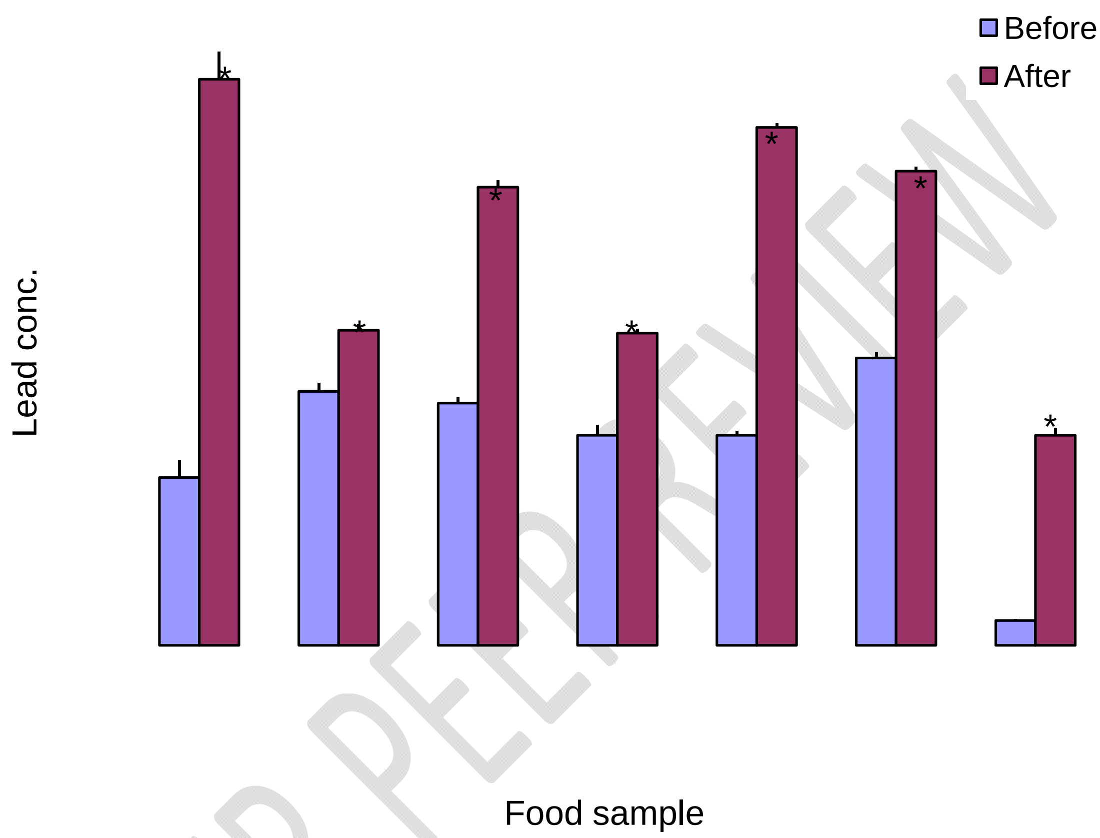

Figure 1: Comparison of concentration of lead before and after exposure in the different food samples.

Values are expressed as mean + SEM, n = 3.
* = significantly different from before exposure at p<0.05

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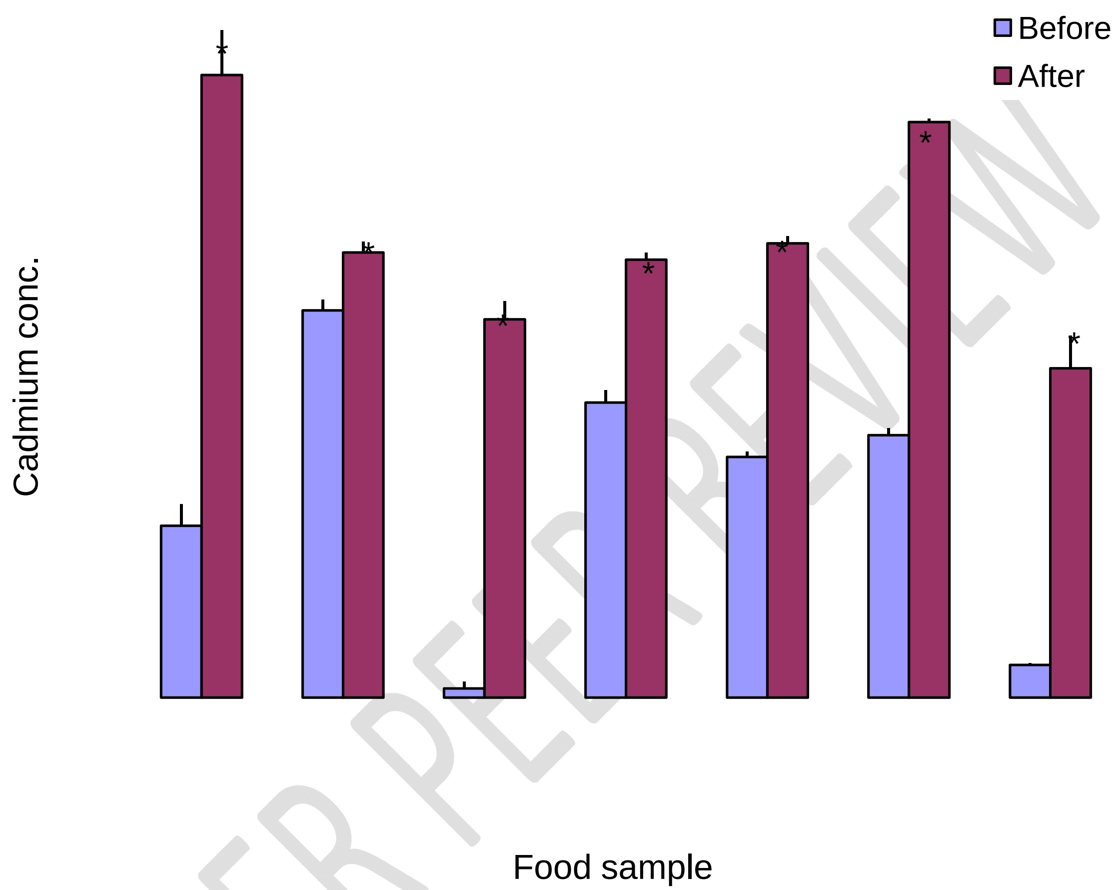

Figure 2: Comparison of concentration of cadmium before and after exposure in the different food samples.

Values are expressed as mean + SEM, n = 3.
* = significantly different from before exposure at p<0.05

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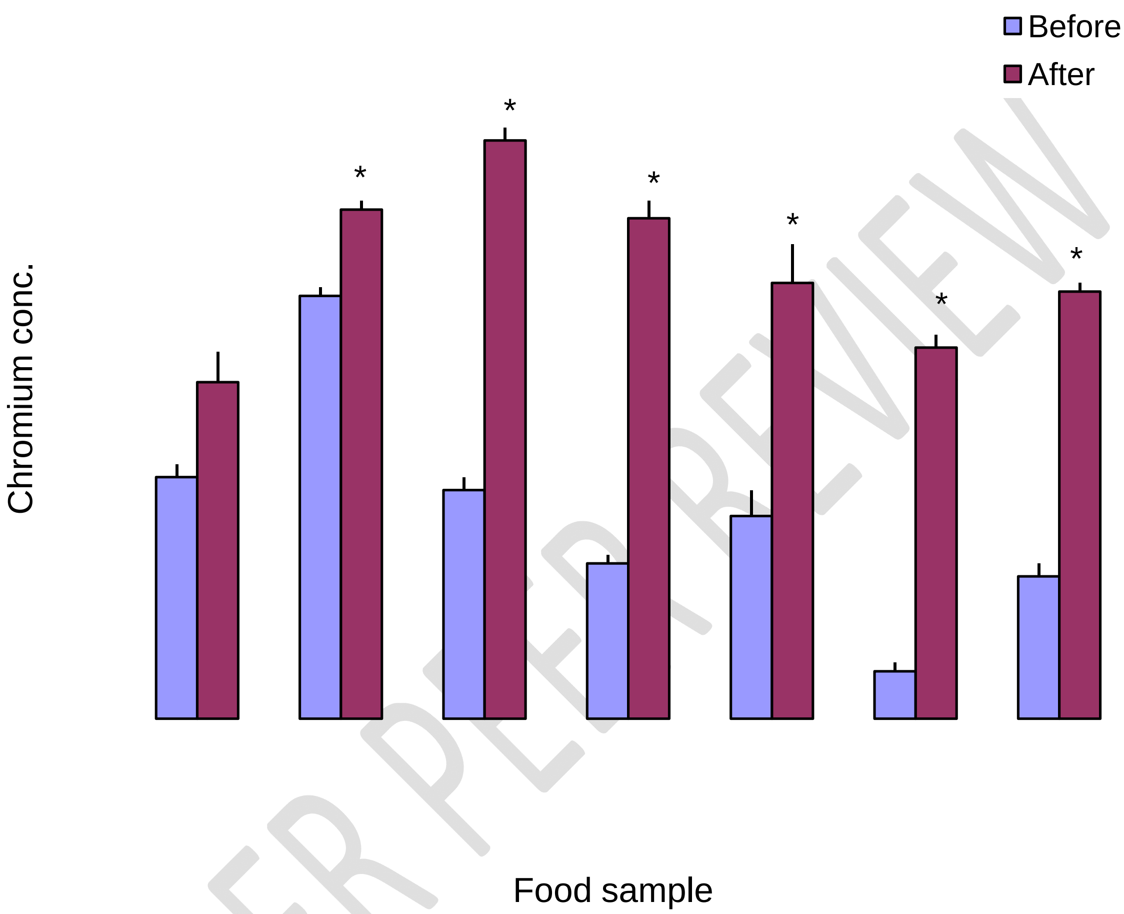

Figure 3. Comparison of concentration of chromium before and after exposure in the different food samples.

Values are expressed as mean + SEM, n = 3.
* = significantly different from before exposure at $p<0.05$

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In this study, the levels of Pb, Cd and Cr were determined in some ready-to-eat foods, including garri, afang soup, melon soup, white rice, beans, stew and meat pie that are sold around the filling station environments in Calabar metropolis. The results obtained, as presented in Figures 1-3, showed that the levels of Pb and Cd were significantly ($p<0.05$) increased in garri, afang soup, melon soup, white rice, beans, stew and meat pie, while the level of and Cr was significantly ($p<0.05$) increased in afang soup, melon soup, white rice, beans, stew and meat pie after 6 hours of exposure to petrol station's atmospheric conditions. This shows that there is high possibility of contamination of most of the ready-to-eat foods sold around the petrol stations evaluated with heavy metals (such as lead, Cadmium and Chromium).

Discussion - **combine discussion with result and references to be sequentially numbered mentioned from introduction, methods and discussion in square bracket**

The presence of heavy metals contaminants in foods has been a serious concern in most societies of the world in recent times. Generally, increased exposure to heavy metal contaminants introduced into the air from industrial activities and traffic congestion is a consequence of advancement in industrialization. Exposure of food and food substances to heavy metals contaminated environments is likely to result in the contamination of such food substances with heavy metals. The petrol stations environment is among the areas reported to be characterized by heavy metal contaminations (Dauda & Odoh, 2012; Afrifa *et al*., 2013). The presence of heavy metals in the air, water and food forms a major health threat globally (Matthew *et al.*, 2002). Such human activities as use of agricultural pesticides, increase in industrialization and mining are known to release high amount of heavy metals into the environment, thereby increasing the levels of heavy metal pollution in the ecosystem (Srikanth *et al*., 2004). Consumption of food items displayed at these environments is therefore likely to expose the consumers to the risk of these heavy metals toxicity. Some heavy metals are known to cause various health hazards to individuals that consume those foods that are enormously contaminated with the metals. Generally, heavy metals get into human systems following consumption of foods and drinking of water that are contaminated with heavy metals. This study is therefore important in determining the possibility of dietary consumption of heavy metals from food sources. This may also play a key role in evaluating food safety and the consequent effects of heavy metals on the consumers.

This study assessed the level of some heavy metals in ready-to-eat foods such as prepared garri, meat pie, stew, rice, beans, afang and melon soups that are sold at the petrol refueling stations in Calabar. It was observed from the results of this study showed that petrol refueling stations atmosphere, introduced high levels of Pb and Cd into garri, afang soup, melon soup, white rice, beans, stew and meat pie sold within premises of the refueling stations. Also, high level of Cr was recorded in garri, afang soup, melon soup, white rice, beans, stew and meat pie at the petrol stations in Calabar, compared to the level recorded for the freshly prepared foods. According to the "Joint FAO/ World Health Organization Expert Committee on Food Additives (JEFCA) the established provisional tolerable weekly intake for lead is 0.025 mg/kg body weight" (JEFCA, 2004). Also, the report of "WHO provisional guideline records 0.01 mg/L as the adopted standard for drinking water" (WHO, 2004). According to the FAO/WHO standard, "the permissible level of cadmium and lead is 0.05 and 0.2mg/kg, respectively".

The level of these heavy metals, particularly Pb, Cr and Cd, were observed to be higher than the World recommended limits. The observed increase in heavy metals contents in ready-to-eat foods at the petrol stations implies that the atmospheric environment of petrol stations in Calabar must have been contaminated with these heavy metals, from where they are likely introduced into these food items. Reem *et al*., (2012) reported that the level of Pb in some brands of chicken liver meat sold in open market in Iraq is higher than the permissible limits. This suggested the possibility of the introduction of Pb from a contaminated open market atmosphere into the meat. Results of the current study gives a clear indication that most ready-to-eat foods sold around the petrol stations may be prone to contamination with heavy metals (such as Pb, Cd and Cr). The results of this study therefore support the report of Dauda & Odoh (2012) and Afrifa *et al*. (2013), that the level of atmospheric air within the petrol refueling stations in South South region of Nigeria are heavily contaminated with heavy metals. Also, Thirulogachandar *et al*., (2014) reported that the presence of heavy metals in poultry liver meat correlates the extent of contamination due to water, foodstuff, air and premixes intake by the chicken.

The results of this study are in agreement with work done by Oladimeji *et al*. (2014) that who reported that ready-to-eat foods at traffic and industrial activities congested areas contain high level of such heavy metals as Cd, Pb and Cr. Since the concentrations of Pb, Cd and Cr in the ready-to-eat foods in this study were high, it can be deduced that consumption of these foods may pose a significant health hazard to their consumers. In a study carried out by Sharma *et al*. (2009), “a concentration of 1.96 mg/kg was recorded for cadmium in tomatoes collected from production and market sites of a tropical urban area of India”. Similarly, high amounts of cadmium was been reported by Jimoh *et al* (2012). However, the studies carried out by Radwan & Salama (2006) in Egypt reported a concentration of 0.01 ± 0.00 mg/kg of Cd (i.e., below the standard threshold) in some Egyptian fruits and vegetables, including tomatoes. Since the level of Cd recorded in this study for ready-to-eat foods was above the reported tolerable limits, consumption of these foods may pose a health risk to the consumers, as observed by Zheng *et al*. (2007).

A study on heavy metal content of Egyptian fruits and vegetables reported the concentration of 0.26 ± 0.09 mg/kg (Radwan & Salama, 2006), while Aryan Dermisbas (2009) reported Pb concentration of 0.43 ± 0.08 mg/kg for tomatoes from market survey in Egypt. It has been reported that the presence of lead in foodstuff at a concentration higher than the maximum permissible limit of 0.2 mg/kg can pose both long and short term health hazard (Oladimeji *et al*., 2014). According to this report, “short-term exposure to high levels of lead can cause brain damage, paralysis (or lead palsy), anaemia and gastrointestinal symptoms”. Consumption of foods containing high concentration of lead may therefore pose a major health challenge(s) to the consumers. Short-term exposure to low amounts of lead has been reported to produce adverse effects on neuro-behavioral development of particularly young children (Food Safety Authority of Ireland, 2009). The results obtained from this present research work suggest that the consumption of ready-to-eat foods exposed to petrol stations environment for 8hours are liable to cause lead toxicity.

The potential health risks that are known to be associated with exposure to heavy metals in foods have attracted the concern of researchers in recent times. Literature reports clearly pointed out that heavy metals are dangerous due to their non-biodegradability property and long biological half lives, hence their ability to bio-accumulate within living tissues (Jarup, 2003; Sathawara *et al*., 2004; Banerjee *et al*., 2011). According to Demirezen & Uruc (2006), consumption of foods contaminated with heavy metals pose a serious health threat to the consumers due to their toxicity effects, bioaccumulation and biomagnifications in food chains. Although it may be difficult to prevent the contamination of the atmospheric air around industrialized areas with heavy metals, it is important that strict measures of controlling the exposure of food items to these contaminated environments should be devised and adopted. Among these measures may include prevention of exposure and sale of ready-to-eat foods in such open contaminated environments as petrol refueling stations. With this, the prevalence of food contamination with heavy metals will be reduced, and the rate of consumption of heavy metals contaminated foods will also be reduced.

Conclusion

From this study, it may be concluded that exposure of ready-to-eat foods at the filling station's atmospheric conditions may cause heavy metal contamination to the foods, particularly Pb, Cd and Cr. It is therefore advisable to protect the ready-to-eat foods sold within and around the filling stations from direct exposure to the atmospheric conditions of the filling stations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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