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Assessment of heavy metals leachability from traditional clay pots "inkono"and "ibibindi" used as food contact materials

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Abstract

The clay pots may transfer ones of their constituents into foodstuffs when they are coated with glazes which are said to contain heavy metals like Pb and Cd. This study was conducted to determine if traditional clay pots (unglazed) can also behave the same way.

Leachate from the clay raw pulp material was initially analysed for heavy metals determination followed by assessment of heavy metals leached from the final product "clay pot". Leaching tests were conducted using acetic acid 4 % and juice from foodstuffs mostly cooked or brewed in clay pots (beans, tomatoes, carrots and banana juice). The concentrations of heavy metals in leachates were determined by flame atomic absorption spectrometry.

By cooking beans in clay pots, the concentrations in leachates were in ranges of 0.385-2.692, 3.654-3.846, 470.000-682.692 and 1.731-1.923 mg/kg for Pb, Cd, Fe and Zn respectively while during cooking tomato-carrot sauce in these clay pots, these concentrations were found in ranges of 0.000-0.192, 1.731-3.076, 1023.077-2005.769 and 0.000-1.923 mg/kg for Pb, Cd, Fe and Zn respecetively. By brewing banana liquor in these clay pots, the concentrations in leachates were respectively evaluated in ranges of 0.224-1.092, 0.000-0.196, 37.676-57.990 and 0.000-2.204 mg/L for Pb, Cd, Fe and Zn.

Results of this study showed that Pb, Cd and Fe were transferred in considerable amounts which exceeded the safe limits in food established by WHO. As heavy metals are toxic in trace concentrations, due to bioaccumulation, traditional clay pots constitute a public health hazard when used as food contact material. However, as the geochemical properties of clay are different from regions to region and the techniques of making them differ, further studies should be undertaken to check the leachability of these heavy metals from different type of pots.

Keywords: *Heavy metals, adsorption, clay, clay pots, leaching, health risk*

1. Introduction

Clay products have been in use for time immemorial. They have been very essential in day-to-day life. For sometimes, it has been thought that the constant use of clay items as food ware in different domestic purposes could be a source of health hazards (Parr et al., 1991). These are particularly due to the migration of heavy metals from their surface into food and beverages cooked or stored therein.

Because of health and sanitation reasons, there are regulations concerning the leaching of toxic elements from containers or utensils that come in contact with foods throughout the world, the total diet study (TDS) sometimes called the market basket study is carried out by many organizations, its aim is to determine levels and various contaminants and nutrients in food for the purposes of estimated intakes. Nothing from food-contact articles should impart flavor, color, odor, toxicity, or other undesirable characteristics to food (USFDA, 2001).

Heavy metals, when they are contained in any cooking material used for making or storing food and beverages, they may migrate (leach) from the material into the food, or beverages under different conditions such as pH, type of food or high temperature reached during cooking. Therefore they may cause acute or chronic poisoning.

The objective of this paper is to assess the leachability of heavy metals from clay material into food and beverages cooked, prepared or stored in.

2. Materials and methods

2.1 Sampling and preparation of clay materials

Different samples which include clays, clay pots and cooked or prepared foodstuffs in clay pots were used to conduct this study. Clays were collected in the middle of rain season in June from Southern Province of Rwanda precisely in two sites located at Nyakanga marsh in Muhanga District and Bishya marsh in Nyanza District. At field, clays were dug at a deep of about 1 m using a shovel. Thus, clays from different corners were mixed in order to homogenize the sample and then, small quantities were collected as sample representatives in polyethylene bags for laboratory analysis. The remaining part of clays was used to make clay pots so that clay samples could have the same composition as clay pots materials (USEPA, 2000).

Clay pots were made by molding clay body, shaping it and finally firing by using fire woods. Besides, different ingredients used in cooking and in preparing foods and beverages in clay pots were purchased at local market.

The clay samples to be analyzed were air- dried in the laboratory, ground in porcelain mortar using a pestle and sieved through a 5 mm stainless sieve(mesh) then stored at room temperature in polyethylene bags for further analysis.

2.2 Extraction of heavy metals in clay samples (leaching)

Analysis of heavy metals in soil takes a few forms and depends on the purposes of analysis. For this study the objective was to determine the bioavailable quantity of heavy metals released when clay material is in contact with different solutions (extractants). This is different from the determination of total elements contents which carried out by performing strong acids digestions. Extractable heavy metals contents in clay samples were determined in leachate solutions obtained after extraction done by using various chemical extractants. It has been found that one extractant can not provide the best correlations for several heavy metals. The extraction methods were selected according to the British and American literature (Sparks, 1996).

Soil extractants listed in Table 1 were used to isolate and extract elements from particular soil phases. According to the concept of Viets, elements occur in soluble, exchangeable, organically complexed, secondary (carbonates, hydroxides of iron and manganese, sulphides, etc.) and primary mineral soil 'pools' of different solubility (Viets, 1962). Reagents of different strength can access one or a few 'pools'. Thus the forms of heavy metals in solution after leaching don't need any form of sample pretreatment for their determination. In order to determine the contribution of reagents (extractants) and the preparative analytical steps to concentrations of metals of concern in clay samples, blank solutions were prepared to evaluate this contribution to error in the measurements. For each extractant, a blank solution of that extractants and water free metals reagent was obtained in the same way as leachate solution.

Table 3: Extractants used for heavy metals leaching in clay samples

Extractants	**Ration:** **soil: extractants (g/mL)**	**Extraction duration (h)**
Deionised water (DW) (pH 5.7)	1 : 10	**24**
1 M NH_4 OAC (pH 7)	1 : 10	**24**
H_2SO_4 (pH 2)	1 : 10	**24**
H_2SO_4 (pH 3)	1 :10	**24**

Procedures:

Heavy metals extraction from clay samples was done by mixing it with different extractants solutions which include deionised water, acetyl ammonia 1M and sulfuric acid (pH 2 and pH 3), in bottles. Mixtures were shaken on laboratory shaker, filtrated and the filtrates as results of leaching were acidified where necessary and stored in polyethylene bottles for further analysis.

Leaching of heavy metals from clay pots were tested by using two approaches: leaching by using simulated food and leaching by using foodstuffs.

By simulated food method, heavy metals leachability were tested using FDA method for determination of heavy metals extracted from ceramic foodware (FDA, 2000). According to Jorhem et al. (2007), 4 % acetic acid solution (almost equivalent to household vinegar) seems to be an appropriate simulated solution for acidic food. In this study, leaching was tested by filling clay pots with 4% acetic acid (pH = 2.7) to within 6-7 mm of overflowing and leaves it for 24 hours at room temperature (20 to 24 ^{0}C).

By foodstuffs, different types of foods were prepared in clay pots as follow:

- Tomato and carrot sauces were cooked according to the traditional recipes in Rwanda .The tomato- carrot sauce as food stuff was chosen because it was found to be a low- pH food stuff (4.4) which is a crucial parameter for leaching.To make the red sauce the following ingredients were used in clay pots of every site: 250g of red tomatoes, 1 onion, and 2 teaspoons of oil. The sauces were cooked for 1 hour on clay oven using wood charcoals as source of heat, then the daily conditions of an average Rwandan home were reproduced and, therefore, it was possible to measure leached contaminants (heavy metals).
- Beans were chosen because of its long cooking time (contact time), but also high temperature. Besides, they are among foods most cooked in clay pots in Rwandan society. To cook these beans, 500g were put in every clay pot filled with water and the cooking took 4 hours on clay oven. Cooked beans were dried in a stove at 110 ^{o}C till to a constant weight. Finally the samples were ground in a mortar in order to be reduced in small pieces (powder). This was stored in acid - washed plastic bottles for preventing contamination until the time it was analyzed.
- Banana liquor (urwagwa**)** is one the most known historical beverages prepared in clay pots in Rwanda culture. It was selected because of its content in alcohol (ethanol). Aqueous – ethanol as a solvent can maximize dissolution of organic and inorganic material during

fermentation and storing (Mathlouthi, 1994). This liquor was prepared according to the traditional recipes in Rwanda: Banana juice purchased at market was poured in clay pots together with sorghum flour which acts as yeast. The juice was fermented for 4 days. When the liquor was obtained, it was stored in acid – washed plastic bottle and preserved at 4 ^{0}c in a fridge for further analysis.

Leachate solutions from clay pots (using simulated food, AC 4 %)

To the same way, the forms of heavy metals in leachates are metal exchangeable forms (ions). We didn't need any pretreatment. To evaluate the contribution of the reagents and the preparative analytical steps to error in the further measurements, a blank of acetic acid 4 % solution of the same volume as leachate was used.

Leachate solutions from clay pots (using foodstuffs)

Beans and carrot - tomato sauce

Because heavy metals can be found in plant tissues, beans and carrot – tomato sauce were decomposed. Acid – wet digestion was chosen as a method for sample decomposition in order to destroy sample matrixes for obtaining accurate and reliable results. The destruction of plants tissue referring to the methods for the determination of Pb, Cd , Fe and Zn with atomic absorption technique (Fred,1989). To evaluate the contribution of heavy metals found in plants tissue before to the amount of heavy metals leached, blank solutions were prepared. To do this, beans and carrot-tomato mixtures not cooked in clay pots were used together with water free metals.

Banana liquor and banana juice samples

Five milliliters of banana liquor of each sample were used. Samples were digested by adding 15 ml of nitric acid (65%) and making it up to 50 ml with deionized water. This was heated until the solutions were fully digested and reduced to 10 ml .The solutions were allowed to cool and then filtered. A blank of deionized water was prepared in the same way for examinating the contribution of chemical reagents (Maduabuchi et al, 2007). In addition, because of expecting the presence of heavy metals in banana juice, this was also used as blank for determination of leached amounts. To prepare banana juice, 20 ml of banana juice were mixed with 10 ml of HNO_3 (65%), then the volume was completed to 100 mL using de-ionized water and the mixture was filtered to remove solid particles (McHard et al, 1976). To evaluate the contribution of chemical regents (contamination), a blank of deionized water was prepared in the same way as the sample solution.

2.3 Determination of pH

Soil pH was determined in a soil – water suspension by using pH- meter. This suspension was made by mixing 10.0 g of finely grounded clay and 25 ml of deionized water in 50 ml beaker. The mixtures were left for 16 hours for equilibrium (PAUWELS et al, 1992)

2. 4 Atomic absorption spectroscopy (AAS) analysis

For quality assurance and quality control, standard and blank solutions for every contaminant of concern were measured before actual heavy metals analysis. All digested samples were submitted to AAS analysis to determine concentrations of Pb, Cd.Fe, and Zn. To do this, Atomic Absorption Spectrophotometer model AA 10 PLUS was used.

3. Results and discussions

3.1 The extractable heavy metal contents in clay samples using chemical extractants

The extractable heavy metals obtained from various chemical extractants were lead, cadmium, iron and zinc. Their quantities are shown in the table 2.

Table 2: The extractable heavy metals contents in clay samples

Site	Metal	Extractants and concentration of metals (mg/L)			
		DW (pH 5.7)	*H_2SO_4 (pH 2)*	*H_2SO_4 (pH 3)*	*NH_4AC (pH 7)*
Nyakanga	Pb	< DL	0.053	< DL	0.100
	Cd	<DL	0.162	0.007	0.005
	Fe	16.100	4.339	0.220	2.588
	Zn	0.407	0.025	0.020	0.047
Bishya	Pb	0.007	0.008	< DL	0.067
	Cd	< DL	0.003	< DL	0.004
	Fe	3.880	0.869	0.128	0.184
	Zn	0.116	0.023	0.018	0.042

DL = value less than detection limit of 0.001 mg/ L

Depending on the surrounding geological environment, clays contain a wide range of heavy metals with different concentrations. This founding was confirmed by the idea of Rimmer (2004), which indicated that the content of the mobile form of heavy metals depends on the nature of metal ion, the nature of extractant and pH.

Note that the extractants power was chosen referring to their leaching properties. Deionised water (pH = 5.7) extracts water soluble pools .Extraction with water takes place either by dissolution or by diffusion through diffuse layer because of high difference in metals concentration between liquid phase (water) and solid phase. For this study they were in a ratio of 1: 10. So heavy metals (exchangeable fractions) tend to migrate from solid phase to the liquid phase till to the equilibrium (Alan, 1993). The water-soluble fraction (i.e. deionised water extractable form) for studied elements follows the order Fe > Zn >Pb and Cd. Our foundings showed that in clay, heavy metals can leach by mere shaking in water and therefore some of the retained metals may become secondary sources of metal pollution. For 1M NH_4AC (pH 7), heavy metal extractability depending on its relatively high concentration and the metal complexing power of the acetate ion, which prevent readsorption or precipitation of released metal ions (Podlesakova et al., 2001). During this study, this reagent has extracted all studied metals. The comparison between the results on H_2SO_4 at pH 2 and H_2SO_4 at pH 3 shows that the extractant's pH has a considerable influence on extractable heavy metals because of clay minerals pH dependent charge. For all heavy metals H_2SO_4 at pH 2 has extracted more quantity than H_2SO_4 at pH 3.

Among the studied heavy metals, the extractability follows the sequence Fe > Zn >Pb>Cd. One can see that iron is the best extractable metal. This comes from the fact that iron is one of the most abundant elements in earth materials.

Figure 1 gives comparison between the strengths of extractants namely, DW, H_2SO_4 (pH_2) H_2SO_4 (pH_3) and NH_4AC (pH_7) on heavy metals. Among these extractants, DW was the best extractant for Fe and Zn while NH_4AC (pH_7) was the best extractant of lead. H_2SO_4 (pH_3) was a poor extractants for all metals studied.

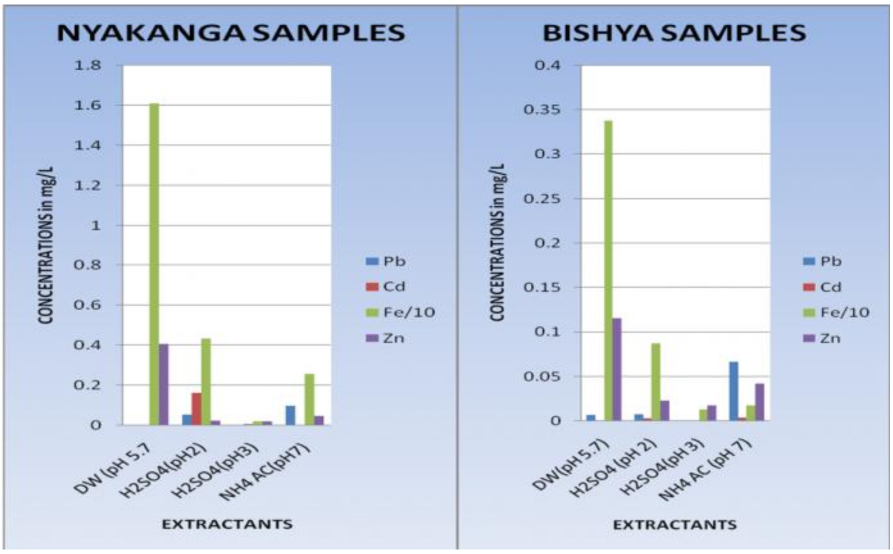

Figure 1: The comparison between the strengths of extractants

3.2 The extractable heavy metal contents in clay samples using foodstuff

3.2.1 The extractable heavy metal contents in clay pots using AAc 4% as simulated food

By referring to FDA method test for determination of heavy metals extracted from ceramic foodware, the extractable heavy metal contents in clay pots found are presented in table 3. Similarly the presented values are the differences between the concentrations in sample and the ones in blanks.

Table 3: Amounts of Pb, Cd, Fe, Zn leached by clay pots by using 4% acetic acid

METAL	*NYAKANGA*	*BISHYA*
Pb (Mg/l)	0.013	0.002
Cd (Mg/l)	0.015	0.005
Fe (Mg/l)	14.53	7. 080
Zn (Mg/l)	0.108	0.008

These results showed that heavy metals can migrate from clay pot material when the latter is tested by FDA method specific to ceramic foodware. Furthermore they show a perfect correlation of extractable heavy metal content in clay pots and the available amounts of these HMs in clays used to make these pots. Again iron is the most leached metal.

There are limits standards of heavy metals leached by ceramic ware when tested by this method. Even the amounts of leached heavy metals found in this research are low comparing to them, these limits concern glazed ceramicware which are most likely to contribute to the dietary intake of heavy metals, because they are coated with glazes containing heavy metals, lead and/or cadmium that is why these two elements are the most concerned when testing ceramicware. (FDA, 2008).

Concerning this study no standards limits have been found for unglazed ceramicware (traditional). However, referring to the known toxicity of these elements even in trace concentrations because of their bioconcentration, the results of this FDA routine method on this study, are high given that these items are not glazed.

3.2.2 Concentrations of leachable Pb, Cd, Fe and Zn using Beans: Tomato –Carrot sauce as foodstuff

Given that the samples of beans and Tomato-carrot sauce were dried during the process of pretreatment for being digested and that the concentrations given by FAAS readings were in mg/l. these concentrations should be expressed referring to dry weight. To do this the following calculations were done. Given N mg/l, the concentration of any given heavy metals among contaminant of concern (COC).By using the quantity of dry sample used during digestion, 1.3 g of sample's dry weight was digested and the final volume was made up to 250ml.The transformation of N mg/l in mg/kg of dry weight was done as follow:

$$1000ml \rightarrow Nmg$$

$$250ml \rightarrow \frac{Nmg \times 250ml}{1000ml} = N \times 0.25mg$$

$N \times 0.25$ was the number of mg found in 250 ml, but it also this number of mg which was in initial 1.3 g of sample's dry weight , that is $N \times 0.25$ mg in 1.3. This amount were expressed as mg of HM in 1 kg of dry weight of sample as follow:

$$1.3g \rightarrow N \times 0.25mg$$

$$1000g \rightarrow \frac{N \times 0.25mg \times 1000}{1.3}(mg/kg)$$

Table 4: Amounts of Pb, Cd, Fe and Zn leached by clay pots in some foodstuffs

Site	Foodstuff	Concentrations (mg/Kg of dry weight)			
		Pb	Cd	Fe	Zn
Nyakanga	Beans	2.692	3.654	682.692	1.923
	Sauce	0.192	1.731	2005.769	1.923
Bishya	Beans	0.385	3.846	470.385	1.731
	Sauce	< DL	3.076	1023.077	<DL

<DL = value less than detection limit of 0.001 mg/L (=0.192mg/kg of dry weight)

Even though test with simulated food is the usual test used to confirm if any given food contact material can transfer its components in food, during this research we have used the foodstuffs to meet the realistic conditions reached when these utensils are used in Rwanda. As can be seen, substantial amounts of COC have been leached from clay pots. Even it is considered to be the least toxic among the COC for this study, once again iron shows significant quantities. This confirms the leaching of heavy metals from clay pot material into foodstuffs cooked in them. With reference to the results obtained for leachable heavy metals in clay samples, these results confirms definitely that leached amount from clay pots is proportion to the present amount in clay sample used to make them. Concerning tomato- carrot sauce the foodstuff used as a low – pH food (its measured pH was 4.4) it seems to be efficient extractant for iron. This can indicate that most of leached iron was bounded to pH dependent charges. Pb, Cd and Zn are also leached. This can be explained by the fact that low acidity is known to influence greatly the leaching since H^+ can be considered as competitive cations (among many others including heavy metal ions) in ion exchange processes (Inglezakis *et al.*, 2003). For beans (pH 6.4), they show also non negligible amounts for both COC. They were used as foodstuff cooked for long time, but also on high temperature. Heavy metal Pb, Cd and Zn show high concentrations comparing to what found when tomato-carrot sauce is used.

3.2.4 Concentrations of leached Pb, Cd, Fe and Zn using banana liquor as foodstuff

According to Mathlouthi(1994), aqueous ethanol mixture is the best extractant , because it interacts with both mineral and organic pools of heavy metals(pollutants) within material, especially by dissolving them. More surprisingly the low measured pH of this foodstuff (pH 3.6) can explain the high leached amounts of COC when banana liquor is used as foodstuff By comparison of banana juice (pH 4.7) from which this liquor was derived, this high acidity can be related to alcohol content formed during fermentation because alcohols are known to be more acidic than alkaline solutions. Besides the long – contact time must be taken in account as leaching influencing parameter because to get banana liquor used in this study the juice was fermented during 4 days.

Table 5: Amounts of Pb, Cd, Fe and Zn leached by clay pots in banana liquor

SITE	CONCENTRATIONS (mg/l)			
	Pb	Cd	Fe	Zn
NYAKANGA	1.092	0.196	57.990	2.204
BISHYA	0.224	<DL	37.676	<DL

<DL = value less than detection limit of 0.001 mg/L

3.3 Comparison between concentrations of leachable heavy metals and recommended maximum levels allowed in food stuffs

Regarding standards limits of migrating toxic elements in foodstuffs from ceramic articles, the existing ones concern the glazed articles. They are specific to population groups (children or adults), to categories of ceramic article but also they are time based (ex: Provisional Tolerable Weekly Intake (PTWI), provisional daily total tolerable intakes (PDTTI); thus the results of this study can't be compared to them. However, the results of the present study have been compared to the Maximum Contaminant Levels (MCL) allowed to enter human body through the food. These are safe limits for toxic elements (EU and WHO safe permissible levels). For the most toxic elements of this study, these limits are 0.05mg/kg for Cd , and 0.1-0.3 mg/kg Pb for (Kumar et al*, 2009.*). Based on the above standards it is shown that clay pots when are used to prepare beans and carrot – tomato sauce leach Pb and Cd levels that exceed their maximum contaminant levels (MCL). Concerning Banana liquor (BL) in which the leached heavy metals can't be expressed in terms of dry weight(only found safe limits) .the concentrations found are almost the same as PTWI. Note that these are Provisional Tolerable Weekly Intake. For lead PTWI =1.5mg/l and 0.1 mg/l for Cd.

Even though iron is not very toxic, its leached amounts are too high because they exceed even the maximum recommended dietary level of 18mg/day. Above the Recommended dietary allowance may result in iron overload (Hemochromatosis) or iron/zinc overload in the population and this may lead to gastroenteritis and abdominal pains which has been reported as consequential cause of human iron and zinc overload (Macrae et al, 1993). As far as zinc is concerned, the results of this study show that the concentrations of leached zinc under different foodstuffs are all under its maximum recommended dietary level (10-15 mg /day).In spite of its low leached amounts, it is well-known that chronic exposure to heavy metals even at relatively low levels can cause adverse effects (Chang, 1996).

4. Conclusions

With regards to the objectives of this research and the results obtained, this study has shown that traditional clay pots used in Rwanda as food contact materials may transfer heavy metals in food stuffs. According to the regulation 1935/2004 of EU applied to food contact materials which state that "all materials intended to be brought into contact with food must not, under normal or foreseeable conditions of use, transfer constituents to food in quantities, that could endanger human health; or bring about an unacceptable change in the composition of the food; or deteriorate the organoleptic characteristics (taste/odor) of the food", tradition clay pots tested in this study transfer in food heavy metals in quantities which exceed safest limits . Thus when they are used as food contact materials they may represent a remarkable dietary intake of heavy metals which constitute a public health hazard.

References

1. Alan wild: soils and environment, sorptive properties of soils; Cambridge university press, 1993, p 55.
2. Ashok Kumar, Sharma I.K., Alka Sharma, Sarita Varshney, and Verma P.S.; Heavy metals contamination of vegetable foodstuffs in Jaipur ; EJEAFChe ; Jaipur (India) ; 2009.
3. BFR (The Federal Institute for Risk Assessment) , Lead and Cadmium from Ceramics, Updated Expert Opinion No. 023/2005 of BfR from 26 March 2004.
4. Fred Kruis; Environmental chemistry, selected analytical chemistry, UNESCO-IHE, Institute for water education, 1989.
5. Inglezakis V.J., Loizidou M.D., Grigoropoulou H.P. Ion exchange of Pb2+, Cu2+, Fe3+, and Cr3+ on natural clinoptilolite: selectivity determination and influence of acidity on metal uptake, Journal of Colloid and Interface Science, 2003, 261, 49-54.
6. Jorhem L., Fjeldal P., Sundström B. and Svensson .K., Lead Extracted from Ceramics under Household Conditions, 2007.

7. Maduabuchi J-M. U., Adigba E. O., Nzegwu C. N., Oragwu C. I., Okonkwo I. P., Orish E. Orisakwe ; Arsenic and Chromium in Canned and Non-Canned Beverages in Nigeria: A Potential Public Health Concern ; International Journal of Environmental Research and Public Health; 2007.
8. Macrae, R.; Robinson, R.K. and Sadler, M.J. Encyclopedia of Food Science, Food Technology and Nutrition. New York Academic Press 3, 1993, 557-565, 4: 2573-2581, 3665-2672.
9. Mathlouthi M., Food packaging and preservation. New York: Chapman and Hall, 1994.
10. McHard J.A., Winefordner J.D. and Attaway J.A.; A new hydrolysis procedure for preparation of fruit juice for trace element Analysis by Atomic Absorption spectroscopy, J. Agric. Food chem., 1976, 24, 41..
11. McMurry J. Fundamentals of Organic Chemistry (Fifth ed). Agnus McDonald., 2003, pp. 409.
12. Pauwels J.M., Van Ranst E.,Verloo M.,Mvondo ZE A. ; Manuel de laboratoire de pédologie : Méthodes d' analyses des sols et de plantes, Equipement, gestion de stock de verrerie et de produits chimiques. publications Agricole-28,1992.
13. Podlesakova E., Nemecek J., Vacha R., Mobility and bioavailability of trace elements in soils. Trace Elements in Soil. Bioavailability, Flux, and Transfer. Edited by I. K. Iskandar and M. B. Kirkham. USA, 2001.
14. Parr R. M., Crawley H., Abdulla M., Iyengar G. V., Kumpulaimen J.; Human Dietary Intakes of Trace Elements: A Global Literature Mainly for the Period 1970–1991, IAEA/NAHRES-12, Vienna, 1991.
15. Ralph E. Grim. Clay mineralogy; second edition. Mc graw- Hill books, p1-2, 1968.
16. Raymond. Yong, Geoenvironmental Engineering : contaminated soil, pollutant fate, and mitigations, CRC press, 2001, p 150.
17. Regulation 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EC. Good Manufacturing Practice, Food Contact & Migrations
18. Rimmer D. , Determination of heavy metal mobile forms by different extraction methods,2004
19. Sparks D.L ., Methods of soil analysis, volume 3, American society of Agronomy and soil science society of America,1996.
20. U.S. Food and Drug Administration (USFDA), Center for Food Safety and Applied Nutrition. (2001, May 25). Compliance program guidance manual, Compliance Program Guide: 7304.019 - Toxic Elements in

Food and Foodware, Imported and Domestic (FY 03 - 05). Washington DC: U.S. Government Printing Office.).

21. U.S. Food and Drug Administration (USFDA), center for food and applied nutrition (2001, April), history of FDA's total diet study.
22. U.S.Food and drug administration (USFDA) compliance program guidance manual, toxic elements in food and foodware, and radionuclides in food - import and domestic. 2008. http://www.foodsafety.gov/~dms/opa2pmnc.html (*accessed* 08/2/2009)
23. Viets C. F. G. Chemistry and availability of micronutrients in soils. J. Agric. Fd. Chem. 1962, 10, 174–178.
24. WHO, Inorganic lead. Environmental Health Criteria No 165. WHO, Geneva, 1995, 25-32 and 215-234.
25. William Mcglynm, choosing and using a pH meter for food products, Food and Agricultural products research and technology center. Oklahoma State University, 1990.
26. http://www.atsdr.cdc.gov/cercla/01list.htm (accessed 09/27/2009).