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Comparative study of some mineral elements in the Human's Milk ,Animal Milk and Ready Milk in Al- Jufra Area

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الاهداء

الي من كان سبب وجودي . . . . والدتي ووالدي

والي كافة افراد اسرتي الذين وقفوا

بجانبي وساعدوني

اهدي ثمرة هذا الجهد المتواضع .

شكر وتقدير

الحمد الله رب العالمين والصلاة والسلام علي سيدنا محمد خاتم الانبياء والمرسلين وعلي اله وصحبه اجمعين .وبعد .

كما اتقدم بالشكر من بعده سبحانه وتعالي الي المشرف علي هذة الرسالة الذي قدم جميع التسهيلات حتى يظهر هذا العمل بالصورة اللائقة وهو :-

الدكتور : المرحوم محمد الطاهر المحبس

وكما اتوجه بالشكر والتقدير الي اعضاء لجنة المناقشة ورئيس قسم الكيمياء الدكتـــــــور : سالم خليل والي اساتذتي اعضاء هيئة التدريس بقسم الكيمياء بأكاديمية الدراسات العليا .

واحمد الله كثيرا ، واشكره شكرا جزيلا ، فما التوفيق إلا من عنده جل وعلا .

وأخير اشكر الدكتور : اسامة حسينو الاستادة : فطيمة التركي وكل من ساهم معي في اظهار هذا الرسالة بصورة متواضعه ولو بكلمة طيبة .

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Calcium	**Ca**
Iron	**Fe**
Cadmium	**Cd**
Lead	**Pb**
Diverse	**div**
Child's	**Ch**
Mothers	**M**

Abstract

In this research some mineral elements; (Calcium, Iron, Cadmium, and Lead) has been studied in milk from different sources; (human, cows, and canned) which collected from Aljufra locality in Libya for comparison between each source. Also the effect of mother feeding system , her age, and her child age on the concentration of above minerals in mother's milk by using a direct simple questioner for the mothers from whom we collect the samples under studying milk, and then it classified according to the age of mother, her child age, and her feeding system into 27 groups, and to be easy for statistic analysis they has been distributed into 9 main groups as an independent variables of mothers and their child ages , and being the system of feeding the mobile phase of the variables, i.e.;(Factorial random samples design) . the samples has been analyses by Atomic Absorption spectrophotometer to Estimate the concentration of the 4 above elements in each kind of milk samples, and the result was as flow:

Higher intake of calcium was(1295ppm) when the mother's age was less than 30 years, and their child's age more than one year, and takes carbohydrates as a food mostly. While the least amount of calcium(83ppm)was at the mother's age was less than 30 years and their child's age between 6months and one year, and takes carbohydrates as a food mostly.

Where in case of iron, the higher intake was(519ppm) when the mother's age was less than 30 years, and their child's age more than one year, and takes carbohydrates as a food mostly. While the least amount of iron(0.4ppm)was at them other's age40yearsand more ,where their child's age between 6months and one year, and takes diverse system of food mostly.

And the higher intake of cadmium (13ppb) was when the mother's age between 30 and 39 years, and their child's age between 6months and one year, and takes carbohydrates as a food mostly . While the least amount of it(0.009ppb)was at them other's age40yearsand more ,where their child's age was less than 6months, and takes proteins as a food mostly.

While the higher intake of lead (29.2ppm) was when the mother's age was less than 30 years, and their child's age between 6months and one year, and takes proteins as a food mostly. While the least amount of it(0.21ppm)was at the mother's age was less than 30years,where their child's ageless than 6months, and takes proteins as a food mostly.

In general, when comparing the milk of mothers and milk of both cows and cans, it was found that the highest intake of calcium and iron(883and 47ppm), respectively, were in the dairy canned, and the least of both(286and 42ppm), respectively, in the milk of mothers. While the highest amounts of cadmium and lead(0.9ppband 1ppm), respectively, found in the milk of mothers and minimum value of each (0.04ppband 0.5ppm), respectively, found in canned mil.

الملخص

في هذا البحث تم دراسة العناصر المعدنية: (الكالسيوم ، الحديد ، الكادميوم ، والرصاص في الحليب من مصادر مختلفة :(حليب الأم ، حليب الأبقار ، وحليب المعلب للأطفال) في منطقة الجفرة بليبيا ، لغرض مقارنه انواع الحليب ، كما تم دراسة أثر تغذيه الأم و عمر كلٍ من الأم والطفل على كمية العناصر المذكورة أعلاه في حليب الأم ، وذلك من خلال جمع معلومات بواسطة أسئلة بسيطة ومباشرة وجهت للأمهات اللائي جمعت منهم عينات الحليب تحت الدراسة ومن ثم تم تقسيم العينات حسب عمر الأم وعمر الطفل ونوع غذاء الأم الى عدد 27 مجموعه عينات, لتسهيل العملية التحليلية تم توزيعها على 9 مجموعات رئيسيه كمتغيرات ثابته لعمر الأم و عمر الطفل وتحريك متغير لنوع الغذاء (بنظام العينات العشوائي).

تم تحليل العينات بواسطة جهاز طيف الامتصاص الذري لمعرفة تركيز كل من العناصر الأربعة أعلاه في كل حالة وكانت النتائج على النحو التالي:

أعلى كميه للكالسيوم كانت(1295 جزء في المليون) عند عمر الأم ألاقل من 30 عام وعمر الطفل أكبر من عام ونظام التغذيه غالب عليه النشويات، بينما أقل كميه للكالسيوم (83 جزء في المليون) كانت عند عمر الأم أقل من 30 عام وعمرا لطفل بين 6شهور وعام كامل، ونظام التغذية غالب عليه النشويات .

أما أعلى كميه للحديد (519جزء في المليون) عند عمر الأم أقل من 30 عام وعمر الطفل أكبر من عام ونظام التغذيه غالب عليه النشويات، بينما أقل كميه للحديد (0.4 جزء في المليون) كانت عند عمر الأم 40 عام فأكثر وعمر الطفل بين 6 شهور وعام كامل، ونظام التغذية غالب عليه التنوع .

بينما أعلى كمية للكادميوم (13جزء في البليون)وجدت عند عمر الأم من 30 الى 39عام وعمر الطفل بين 6 شهور و عام ، ونظام التغذيه غالب عليه النشويات، بينما أقل كميه للكادميوم (0.009 جزء في البليون) كانت عند عمر الأم 40 عام فأكثر وعمر الطفل أقل من 6 شهور ، ونظام التغذية غالب عليه البروتين .

في حين إن أعلى كمية للرصاص (29.2 جزء في المليون) وجدت عند عمر الأم أقل من 30 عام وعمر الطفل بين 6 شهور و عام كامل، ونظام التغذيه غالب عليه البروتين، بينما أقل كميه للرصاص (0.21 جزء في المليون) كانت عند عمر الأم أقل من 30 عام وعمر الطفل أقل من 6 شهور ، ونظام التغذية غالب عليه البروتين .

وبصورة عامه عند المقارنة بين ألبان الأمهات وألبان كلٍ من الأبقار والمعلبات،وجد أن أعلى كميه للكالسيوم والحديد (883 و 47 جزء في المليون)على التوالي كانت في الألبان المعلبة ، و أقلها (286 و 42 جزء في المليون) على التوالي في لبن الأمهات. أما أعلى كميات للكادميوم والرصاص (0.9 جزء في البليون و 1جزء في المليون) وجدت في ألبان الأمهات وأقلاها (0.04 جزء في البليون و 0.5 جزء في المليون) وجدت في الألبان المعلبه.

Chapter one
Introduction

1.1 Introduction

Milk is considered as a nearly complete food where it is a good source of protein, fat and major minerals. Milk and milk products are main constituents of the daily diet, especially for vulnerable groups such as infants, school age children and old age{1}. It is an ideal source of macronutrients such as calcium (Ca), Potassium (K), and Phosphorous (P) and micronutrients such as Copper (Cu), Iron (Fe), Selenium (Se), and Zinc (Zn). These are known to be essential for normal growth{2}. These metals are co-factors in many enzymes and play an important role in many physiological functions of man and animals. Lack of these metals cause disturbances and pathological conditions{1} . This fact is particularly true in the case of early childhood, because milk is the only source of nutrients during the first months of a baby's life and the diet of growing children contains a high proportion of milk and milk products. An appropriate intake of milk is also recommended for adults as a source of calcium to retain bone mass so that fractures and osteoporosis can be prevented. The trace elements in milk are of interest because of their essential or toxic nature. For instance, Ca, Fe, Ni, and Zn are essential but may become toxic at higher levels, while Pb and Cd are toxic and can be cumulative. Cd and Pb are amongst the elements that have caused most concern in terms of adverse effects on human health {3}. This is because they are readily transferred through food chains and are not known to serve any essential biological function. Children have been shown to be more sensitive to Cd and Pb than adults and the effects are cumulative, the elements build up in the tissues. As a result, the regular absorption of small amounts of elements such as Pb and Cd may cause serious effects on the health of growing children, including retardation of mental development (e.g. reading and learning disabilities) and deficiencies in concentration, adverse effects on kidney function, blood chemistry and the cardiovascular system, as well as hearing degradation{4}. Heavy metals that contaminate the environment and cattle's feed could be excreted to milk at various level, causing a serious problem to cattle and human {1}. Even though the entrance of heavy metals in food chain particularly due to environmental pollution is certain and has well documented health impact to human beings, there is hardly any study report pertaining to the determination of lead, Cadmium, Chromium, Nickel, Iron and Zinc concentration of milk .Thus the level of minerals in the milk must be analyzed, determined, reported.

Determination of the level of Lead, Cadmium, Calcium, and Iron in milk is the basis of this research. The levels will be compared with international standards reported elsewhere. This thesis is divided in to Five chapters as introduction, literature review, materials and methods, results and discussion, and conclusion and recommendation .

1.2-Objectives:

1.2.1-General objectives:

The general objective of the present study is to determine the level of some of essential and toxic minerals in animal's milk(cow's milk), human milk, and canned milk, in AlJufra area.

1.2.2-Specific Objectives:

To determine the levels of some toxic mineral elements in milk .

To indicate health risks and toxic effects of Pb, Cd, Ca, and Fe.

To study the relationship between the concentration levels of mineral elements in milk, and feeding system , and sours of milk.

1.3-The importance of the study:

the importance of this study is try to prevent the damage of the toxic mineral elements existed is in milk.

Chapter two
Literature Review

2.1.Milk and its nutritional importance:

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to the baby and can reduce the risk of many diseases in the baby.

Milk derived from cattle species is an important food. It has many nutrients. The precise nutrient composition of raw milk vary by species and by a number of other factors, but it contains significant amounts of saturated fat, protein and calcium as well as vitamin C. Cow's milk has a pH ranging from 6.4 to 6.8, making it slightly acidic{5}.

Throughout the world, there are more than 6 billion consumers of milk and milk products, the majority of them in developing countries. Over 750 million people live within dairy farming households. World's dairy farms produced about 720 million tonnes of milk in 2011{6}.

Table 2.1: Top ten per capita cow's milk and cow's milk products consumers in 2006:

Country	Milk (liters)	Cheese (kg)	Butter (kg)
Finland	183.9	19.1	5.3
Sweden	145.5	18.5	1.0
Ireland	129.8	10.5	2.9
Netherlands	122.9	20.4	3.3
Norway	116.7	16.0	4.3
Spain	119.1	9.6	1.0
Switzerland	112.5	22.2	5.6
United Kingdom	111.2	12.2	3.7
Australia	106.3	11.7	3.7
Canada	94.7	12.2	3.3

Minerals or milk salts, are traditional names for a variety of cations and anions within bovine milk. Calcium, phosphate, magnesium, sodium, potassium, citrate, and chlorine are all included as minerals and they typically occur at concentration of 5–40 mm. The milk salts strongly interact with casein, most notably calcium phosphate. It is present in excess and often, much greater excess of solubility of solid calcium phosphate{7}.

Milk is known as an excellent source of Ca (1125.76 to 2 019.04 mg/kg), and it can supply moderate amounts of Mg (136.02–196.67 mg/kg), smaller amounts of Zn (0.614-3.163 mg/L), and very small amounts of Fe (0.682-1.93mg/kg) {8}. Breast milk is known to be the best nutrient for the first 6 months of life, and it has unique properties; one is the variability of the compounds in ratio according to the baby's needs for every lactation period,. The powder milk mainly containing 0.001ppm of cadmium and 0.16ppm of lead, where the cow's milk containing 0.07(mg/kg) of cadmium and 0.04(mg/kg) of lead{9}.

2.1.1. Breastfeeding and its importance for grow of children:

Breastfeeding is feeding of an infant or young child with breast milk directly from female human breasts (i.e., via lactation) rather than from a baby bottle or other container. Babies have a sucking reflex that enables them to suck and swallow milk. It is recommended that mothers breastfeed for six months or more, without the addition of infant formula or solid food. After the addition of solid food, mothers are advised to continue breastfeeding for at least a year, and can continue for two years or more. Human breast milk is the healthiest form of milk for babies{10}.

For breastfeeding of women long-term health benefits include:

- Less risk of breast cancer, ovarian cancer, and endometrial cancer.
- A 2009 study indicated that lactation for at least 24 months is associated with a 23% lower risk of coronary heart disease.
- Breastfeeding diabetic mothers require less insulin.
- Reduced risk of metabolic syndrome.
- Reduced risk of postpartum bleeding.
- According to a Malmo University study published in 2009, women who breast fed for a longer duration have a lower risk for contracting rheumatoid arthritis than women who breast fed for a shorter duration or who had never breast fed{11}.

2.1.2. Food environment mother physiological activity and their impact on value of milk nutrition:

As an excretion of the mammary gland, milk can carry out numerous Toxic substances (pesticides, disinfectants, drugs, metals and various environmental contaminants), which constitute a technological risk factor for dairy products {12}. Lead, cadmium and mercury residues in milk are of particular concern because milk is largely consumed by infants and children {13}.

Studies in determination of Pb concentration in cow milk conducted by a number of authors investigated the effect of animal feed, water and locality on the level of lead concentrations. Pb levels in cow's milk from lead-contaminated areas in Croatia were reported in the range 2.1–82.7 _g/l with median value 14.9 g/l, while those in the control group were in the range 1.2–8.2 _g/l with median value 3.1 g/l. The same study shows that effect of animal feed on concentration of cadmium and lead in milk, the values that a lower level except when animals have consumed contaminated feed{14}.

Another study by {12}. levels of toxic metals (Cd and Pb) in milk from different regions of Italy reported. the lowest levels were those of Cd 0.02, range < 0.01–22.80 µg/kg, which were below the instrumental detection limit in almost all the samples (92.5% of non detected samples). The quite low concentration of Cd in milk has been attributed to sequestration of Cd by metallothionein. Pb levels were contained in all samples in concentrations ranging from 0.10 to 9.92 µg/kg and 1.32 µg/kg.

The rate of lead increases by 21.5% in Breast milk of mothers who smoke or live with smokers,{15}.

Some studies made evidence quantifying the concentration of heavy metals in cattle fed. {16}.reported the Pb concentrations in fodders collected from five different pastures varied from 0.034 mg/g to 0.069 mg/g in the leaves and from 0.040 mg/g to 0.065 mg/g in pods for Pb. Level of contamination of fodder with heavy metals as reported in literatures is indicated in Table 2.2:

Table 2.2: Lead and cadmium concentration in dairy cow feed:

Fodder type	Sample number	Cadmium(ppm)		Lead(ppm)	
		Range	Mean ± SE	Range	Mean ± SE
Corn silage	4	0.125-0.30	0.18±0.04	0.428-2.13	0.88±0.17
Straw	4	0.055-0.20	0.12±0.03	0.070-0.40	0.26±0.08
Cattle feed	4	0.107-6.330	2.02±1.46	0.228-1.97	0.90±0.52
Grass	4	0.135-0.190	0.158±0.017	0.250-4.10	2.03±0.95

Also in case of human the presents of lead is different according to the source of food and if it’s fresh or canned ,which is indicated in table 2.3:

Table 2.3; the value of lead in different sources of food and if it’s fresh or canned:

Source of food	The average of lead content (mg/ kg)	
	Fresh	Canned
Juice	0.04	0.24
Legumes	0.02	0.21
Cereals	0.01	0.22
Beef meat	0.23	o.20
Milk	0.03	0.05

{17}.

2.2. Heavy metals:

Heavy metals represent a large group of chemical elements (> 40) with atomic mass > 50 carbon units {18}.Although there is no clear definition of what a heavy metal is, density is most cases taken to be the defining factor. Heavy metals are thus commonly defined as those having a specific density of more than 5g/cm{19}, and that are capable of forming polyvalent captions {20}{21}.

Trace elements, include they are all the elements in the periodic system except bulk elements and macro minerals, they are classified in to two categories: those elements whose essentiality has been established by accepted standards and those for which proof of essentiality does not exist. By the simplest definition an element is essential when it meets the following criteria (i) the element must be present in all tissues of a given animal; (ii) the concentration in a given tissue must be fairly constant from one animal to the other (iii) its withdrawals from the diet must lead to a specific deficiency syndrome; (iv) the deficiency syndrome must be associated with pertinent biochemical changes; (v), both the syndrome and the biochemical changes must be prevented or corrected by administration of the element. Based on the above criteria, V, Mn, Fe, Co, Cu, Ni, Zn and Mo are now considered essential animals. These metals are an indispensable part of bimolecular{22}.These are essential micronutrients with alumna requirement of no more than a few milligrams per day. However,

micronutrients may become harmful when their ingestion rates are too high [23]. In addition, a metal can be toxic or be a micronutrient depending on its chemical form or speciation (e.g. Cv-VI and Cr-III) [22].
Other elements have not proven essential functions in humans and are likely to have adverse physiological effects. Cadmium (Cd), Mercury (Hg), and Lead (Pb) are well known as toxic even when taken in small amount and have became serious environmental hazards for plants, animals and man. They are extremely persistent in the environment, they are non biodegradable and non thermo-degradable in to less harmful constituents and thus their accumulation readily reaches to toxic levels [24].

2.3.Essential metals:

The health and vitality of human beings depends on diets that include adequate amounts of minerals to promote effective physiological processes including reproduction, immune response, brain and other neural functions, and energy metabolism. These elements are essential; they cannot manufactured by the human body and must be obtained through dietary means. Minerals are inorganic elements which do not originate in animal or plant life but rather from the earth's crust. Although minerals make up only a small portion of body tissues, they are essential for normal growth and functioning. Very small quantities of minerals are needed for health and these are measured in micrograms or milligrams, thus supporting their description as micronutrients .Deficiencies of most micronutrients are known to have devastating health consequences. The adverse effects of micronutrient deficiencies are most affect for children, pregnant women, and the fetus[25].

2.4. Toxic metals:

can be accumulated in soils. The elements accumulated in soils are transferred to edible plants, and when animals are fed with these plants; they accumulate high levels of these toxic metals in their bodies to the milk [26]. Although contamination of animal feed by toxic metals cannot be entirely avoided There is a clear need for such contamination to be minimized, with the aim of reducing both direct effects on animal health and indirect effects on human health. The major sources of heavy metal accumulation in to the soil and then to animal feed are rock weathering, vehicular emissions, cattle manure, waste water irrigation, industrial process[3].
Heavy metals disrupt metabolic function by two basic ways[20] First, they accumulate and thereby disrupt function in vital organs and glands such as the heart, brain, kidneys, bone, liver, etc Second, they displace vital nutritional minerals for example, enzymes are catalysts for virtually every biochemical reaction in all life-sustaining processes of metabolism. But instead of calcium being present in an enzyme reaction, lead or cadmium may be there in its place . Toxic metals can't fulfill the same role as the nutritional minerals, thus their presence becomes critically disruptive to enzyme activity Thus, although the basic role of nutritionally essential metals is to provide some components of a vital biochemical enzymatic reaction, the interactions between nutritionally essential and nonessential toxic metals may reduce the health hazard of the toxic metal[27].Toxicity the metals and is closely related to age route of exposure, level of intake, solubility, metal oxidation state, retention percentage, duration of exposure, frequency of intake, absorption rate and mechanisms and efficiency of excretion [1].

Plants are important components of ecosystems as they transfer elements from a biotic into biotic environments. The primary sources of elements from the environment to plants are: air, water and soil. Crops can take up toxic elements through their roots from contaminated soils, and even leaves .They absorb toxic elements deposited on the surface {28}.there by transferred in to primary consumers In addition, the potential they accumulate in different animal source foods such as meat and milk is high {24}. Some studies made evidence quantifying the concentration of heavy metals in cattle fed{16}. reported that Pb, Ni, and Cr concentrations in fodders collected from five different pastures varied from 0.034 mg/g to 0.069 mg/g in the leaves for Pb. The mechanism of heavy metal toxicity is not specifically known but in general lead, mercury, cadmium, and other heavy metals have a high affinity for sulfhydryl (-SH) groups, inactivating numerous enzymatic reactions, amino acids, and sulfur-containing antioxidants, with subsequent decreased oxidant defense and increased oxidative stress. Both bind to metallothionein and substitute for zinc, copper, and other trace metals reducing the effectiveness of metallo enzymes {29}.

2.5. The mineral elements:

2.5.1. Calcium:

Calcium, the most abundant mineral in the body, can be found in some foods, added to others, available as a dietary supplement, and present in some medicines (such as antacids). Calcium is required for vascular contraction and vasodilatation, muscle function, nerve transmission, intracellular signaling and hormonal secretion, though less than 1% of total body calcium is needed to support these critical metabolic functions .Serum calcium is very tightly regulated and does not fluctuate with changes in dietary intakes; the body uses bone tissue as a reservoir for, and source of calcium, to maintain constant concentrations of calcium in blood, muscle, and intercellular fluids .

The remaining of 99% of the body's calcium supply is stored in the bones and teeth where it supports their structure and function. Bone itself undergoes continuous remodeling, with constant resorption and deposition of calcium into new bone. The balance between bone resorption and deposition changes with age. Bone formation exceeds resorption in periods of growth in children and adolescents, whereas in early and middle adulthood both processes are relatively equal. In aging adults, particularly among postmenopausal women, bone breakdown exceeds formation, resulting in bone loss that increases the risk of osteoporosis over time .

The Food and Nutrition Board (FNB) established Recommended Dietary Allowance (RDAs) for the amounts of calcium required for bone health and to maintain adequate rates of calcium retention in healthy people. They are listed in Table(2.4) in milligrams (mg) per day.

Table 2.4: Recommended Dietary Allowances (RDAs) for Calcium				
Age	**Male**	**Female**	**Pregnant**	**Lactating**
0–6 months*	200 mg	200 mg		
7–12 months*	260 mg	260 mg		
1–3 years	700 mg	700 mg		
4–8 years	1,000 mg	1,000 mg		
9–13 years	1,300 mg	1,300 mg		
14–18 years	1,300 mg	1,300 mg	1,300 mg	1,300 mg
19–50 years	1,000 mg	1,000 mg	1,000 mg	1,000 mg
51–70 years	1,000 mg	1,200 mg		
71+ years	1,200 mg	1,200 mg		

Milk, yogurt, and cheese are rich natural sources of calcium and are the major food contributors of this nutrient to people in the United States. Nondairy sources include vegetables, such as Chinese cabbage, kale, and broccoli. Spinach provides calcium, but its bioavailability is poor. Most grains do not have high amounts of calcium unless they are fortified; however, they contribute calcium to the diet because they contain small amounts of calcium and people consume them frequently. Foods fortified with calcium include many fruit juices and drinks, tofu, and cereals{30}.

The two main compounds of calcium in supplements are carbonate and citrate. Calcium carbonate is more commonly available and is both inexpensive and convenient. Due to its dependence on stomach acid for absorption, calcium carbonate is absorbed most efficiently when taken with food whereas calcium citrate is absorbed equally well when taken with or without food{31}.Calcium citrate is also useful for people with achlorhydria, inflammatory bowel disease, or absorption disorders. Other calcium forms in supplements or fortified foods include gluconate, lactate, and phosphate. Calcium citrate malate is a well-absorbed form of calcium found in some fortified juices {32}.

In the United States, estimated calcium intakes from both food and dietary supplements are provided by the National Health and Nutrition Examination Survey (NHANES), 2003–2006. Mean calcium intake levels for males ranged from 871 to 1,266 mg/day depending on life stage group; for females the range was 748 to 968 mg/day. Groups falling below desirable intakes include girls aged 9–13 years and 14–18 years, women aged 51–70 years, and both men and women older than 70 years{33}. Overall, females are less likely than males to get recommended intakes of calcium from food {34}.

Inadequate intakes of dietary calcium from food and supplements produce no obvious symptoms in the short term. Circulating blood levels of calcium are tightly regulated. Hypocalcaemia results primarily from medical problems or treatments, including renal failure, surgical removal of the stomach, and use of certain medications (such as diuretics). Symptoms of hypocalcaemia include numbness and tingling in the fingers, muscle cramps, convulsions, lethargy, poor appetite, and abnormal heart rhythms{35}.

Over the long term, inadequate calcium intake causes osteopenia which if untreated can lead to osteoporosis. The risk of bone fractures also increases, especially in older individuals. Calcium can also cause rickets, though it is more commonly associated with vitamin D deficiency .

Excessively high levels of calcium in the blood known as hypocalcaemia can cause renal insufficiency, vascular and soft tissue calcification, hypercalciuria (high levels of calcium in the urine) and kidney stones . However, hypocalcaemia rarely results from dietary or supplemental calcium intake; it is most commonly associated with primary hyperparathyroidism or malignancy{30}.

High calcium intake can cause constipation. It might also interfere with the absorption of iron and zinc, though this effect is not well established. High intake of calcium from supplements, but not foods, has been associated with increased risk of kidney stones{36}. Some evidence links higher calcium intake with increased risk of prostate cancer, but this effect is not well understood, in part because it is challenging to separate the potential effect of dairy products from that of calcium .

The Tolerable Upper Intake Levels (ULs) for calcium established by the Food and Nutrition Board are listed in Table2.5 in milligrams (mg\ day). Getting too much calcium from foods is rare; excess intakes are more likely to be caused by using calcium supplements. NHANES data from 2003–2006 indicate that approximately 5% of women older than 50 years have estimated total calcium intakes (from foods and supplements) that exceed the UL by about 300–365 mg {33}.

Table 2.5: Tolerable Upper Intake Levels (ULs) for Calcium

Age	Male	Female	Pregnant	Lactating
0–6 months	1,000 mg	1,000 mg		
7–12 months	1,500 mg	1,500 mg		
1–8 years	2,500 mg	2,500 mg		
9–18 years	3,000 mg	3,000 mg	3,000 mg	3,000 mg
19–50 years	2,500 mg	2,500 mg	2,500 mg	2,500 mg
51+ years	2,000 mg	2,000 mg		

2.5.2. Iron:

Iron, one of the most abundant metals on Earth, is essential to most life forms and to normal human physiology. Iron is an integral part of many proteins and enzymes that maintain good health. In humans, iron is an essential component of proteins involved in oxygen transport {37}. It is also essential for the regulation of cell growth and differentiation {38}. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity {39,40}. On the other hand, excess amounts of iron can result in toxicity and even death {41}.
Almost two-thirds of iron in the body is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues. Smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to muscle, and in enzymes that assist biochemical reactions. Iron is also found in proteins that store iron for future needs and that transport iron in blood. Iron stores are regulated by intestinal iron absorption {42}.
Iron absorption refers to the amount of dietary iron that the body obtains and uses from food. Healthy adults absorb about 10% to 15% of dietary iron, but individual absorption is influenced by several factors {43,44}.
Storage levels of iron have the greatest influence on iron absorption. Iron absorption increases when body stores are low. When iron stores are high, absorption decreases to help protect against toxic effects of iron overload {45}. Iron absorption is also influenced by the type of dietary iron consumed. Absorption of home iron from meat proteins is efficient. Absorption of home iron ranges from 15% to 35%, and is not significantly affected by diet {44}. In contrast, 2% to 20% of non home iron in plant foods such as rice, maize, black beans, soybeans and wheat is absorbed {46}. Nonheme iron absorption is significantly influenced by various food components{47,48}.
Meat proteins and vitamin C will improve the absorption of non home iron {49,50}. Tannins (found in tea), calcium, polyphenols, and phytates (found in legumes and whole grains) can decrease absorption of non home iron {49,50}. Some proteins found in soybeans also inhibit non home iron absorption {51}. It is most important to include foods that enhance non home iron absorption when daily iron intake is less than recommended, when iron losses are high (which may occur with heavy menstrual losses), when iron requirements are high (as in pregnancy), and when only vegetarian non home sources of iron are consumed.
Recommendations for iron are provided in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine of the National Academy of Sciences. *Dietary Reference Intakes* is the general term for a set of reference values used for planning and assessing nutrient intake for healthy people. Three important types of reference values included in the DRIs are *Recommended Dietary Allowances* (RDA), *Adequate Intakes* (AI), and *Tolerable Upper Intake Levels* (UL). The RDA recommends the average daily intake that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in each age and gender group. An AI is set when there is insufficient scientific data available to establish a RDA. AIs meet or exceed the amount needed to maintain a nutritional state of adequacy in nearly all members of a specific age and gender group. The UL, on the other hand, is the maximum daily intake unlikely to result in adverse health effects. Table 2.6 lists the RDAs for iron, in milligrams, for infants, children and adults.

Table 2.6: Recommended Dietary Allowances for Iron for Infants (7 to 12 months), Children, and Adults				
Age	**Male (mg/day)**	**Female (mg/day)**	**Pregnant (mg/day)**	**Lactating (mg/day)**
7–12 months*	11	11	N/A	N/A
1 –3 years	7	7	N/A	N/A
4– 8 years	10	10	N/A	N/A
9–13 years	8	8	N/A	N/A
14–18 years	11	15	27	10
19–50 years	8	18	27	9
51+years	8	8	N/A	N/A

Healthy full term infants are born with a supply of iron that lasts for 4 to 6 months. There is not enough evidence available to establish a RDA for iron for infants from birth through 6 months of age. Recommended iron intake for this age group is based on an Adequate Intake (AI) that reflects the average iron intake of healthy infants fed breast milk. Table 2.7 lists the AI for iron, in milligrams, for infants up to 6 months of age.

Table 2.7: Adequate Intake for Iron for Infants (0 to 6 months)

Age (months)	**Males and Females (mg/day)**
0 to 6	0.27

Iron in human breast milk is well absorbed by infants. It is estimated that infants can use greater than 50% of the iron in breast milk as compared to less than 12% of the iron in infant formula .The amount of iron in cow's milk is low, and infants poorly absorb it. Feeding cow's milk to infants also may result in gastrointestinal bleeding. For these reasons, cow's milk should not be fed to infants until they are at least 1 year old [37]. The American Academy of Pediatrics (AAP) recommends that infants be exclusively breast fed for the first six months of life. Gradual introduction of ironenriched solid foods should complement breast milk from 7 to 12 months of age. Infants weaned from breast milk before 12 months of age should receive iron-fortified infant formula [52]. Infant formulas that contain from 4 to 12 milligrams of iron per liter are considered iron-fortified [53].

2.5.3. Cadmium:

Cadmium is considered to be one of the most toxic metals [1]. It is a typical example of a cumulative poison. The first health effect of cadmium (Cd) was lung damage, reported in workers in the 1930's [54]. Prolonged increased uptake of cadmium interferes with the function of the kidneys and it is the target organ for damage and, when the concentration of cadmium in the renal cortex reaches approximately (200µg/g), damage to the proximal renal tubules occurs and Calcium, phosphorous, glucose, amino acids, and small peptides are lost in the urine[55,56]. Once Cd accumulates in tissues it cannot be removed safely by chelating therapy without causing kidney damage [55].
Cadmium affects calcium metabolism and skeletal changes resulting from calcium loss and ends in a decrease bone mineral density and also in animals cadmium causes softening of bones [54,56].

2.5.4. Lead:

Lead is toxic to the blood and the nervous, urinary, gastric and genital systems[57]. Furthermore, it is also implicated in causing carcinogenesis, mutagenesis and teratogenesis in animal experiments [1]. Lead exposure can cause anemia because it inhibits the body's ability to make hemoglobin. Several studies have also shown an association between lead exposure and increases in blood pressure. Lead readily crosses the placenta and there is evidence that exposure to high levels increases the risk of spontaneous abortion, miscarriage and stillbirth. There is also evidence of less strength that fetal lead exposure can increase the risk of reduced birth weight and premature birth. Human previous studies indicate that lead may have toxic effects on sperm starting at Pb levels of around 40 µg/dL [55].

Children and fetuses are particularly more susceptible than adults, because their bodies are smaller, so the same dose of lead that an adult receives would have a greater effect on a child Age-dependent differences in toxicological response can be due to differences in kinetics and be both quantitative and qualitative Also the toxicological response can depend on the development of receptors to various chemicals, which are developed at various stages. The brain is especially vulnerable during the brain growth spurt, which is the period when the brain undergoes several fundamental changes, like dendritic and axonal growth, synaptogenesis, rapid myelination, etc[58].

In children, even low levels of exposure can result in reduced IQ, learning disabilities, attention deficit disorders, behavioral problems, stunted growth, hyperactive behavior, impaired hearing, and kidney damage. The incidence of childhood cancer has increased, especially for acute lymphocytic leukemia, brain tumor and Wilm’s tumor. The last one is suspected to be in relation with paternal exposure to lead [59]. At high levels of exposure, a child may become mentally retarded, fall into a coma, and even die from lead poisoning. According to recent studies, intellectual impairment in children has been seen at blood lead levels lower than 10 _g/dL [60]. A level of concern for children is a lead concentration exceeding 10 μg/dL of blood. For adults, the level of concern is a concentration above 25 μg/dL. However, there is recent research that indicates that there are subtle effects on children even at levels lower than 10 μg/dL [57].

Chapter Three materials, sampling and experimental

3.1-The materials:

3.1.1- Chemicals Used:.

No.	Name	Formula	Concentration	Company
1	Nitric acid	HNO_3	65%	Carlo Erba.
2	Hydrogen peroxide	H_2O_2	30%	Merck
3	Distilled water	H_2O	---	---

3.1.2-glass wear and Instruments :

1- **Glas wear :**

Pipette, measuring flask (25ml), conical flasks, test tubes, and funnel.

2- **Instruments:**

Heating mental, analytical balance, filter papers, andAtomic Absorption spectrophotometer.

3.2- Methods:

3.2.1- Collection of samples

92 samples of milk were collected from the mothers of the Alafia hospital _ Houn , cow's of Alafia’s breeder's farms, and the manufactured milks were collected from Al- Jufra pharmacies, the samples were took about 2 months for collection .

Some information's about mothers deals with their age , their childe age's ,and their food system has been taken by a simple questioner . the samples were taken by using abreast pump or by manual method and they placed in small closed plastic containers, and keptfrozenatabout-20°Cuntil they analyzed .

There was some problems has being faced during the compilation of the sample switch lack of sufficient awareness among many of mothers.

3.2.2- The Chemical analysis:

The samples has been weighted by analytical balance, and then digested by added 7ml nitric acid , and 7ml Hydrogen peroxide ,then left at room temperature for one day, the digested sample was heated at 60°C for 90min , and then filtrated by filter paper , and diluted twice with distilled water to 25ml.

The Above sample was analyzed by(A.A.S)to (Pb, Fe, Cd)and(Ca) .

The result has been collected, disrepute in tables according to the sores of sample , then statistically analyzed, and finally shown on carves.

3.2.3- Plane of treatments:

There was 29 type of treatments according to 3 independent variables related to mother's milk ; (age of mother, age of the child, and the type of food of milk's sources mothers), and the other milk's sources; (cows and industrial), to determine 4 type of dependent variables; (Ca, Fe, Cd, and Pb) which was shown in tables and carves as a result of 29 experiments (fig.3.1).

Industrial milk ← **Mother's milk** → **Cow's milk**

The age of mothers variables:

Less than 30 years ← **30 -39 years** → **40 years and above**

(M.< 30Y) (M. 30-39Y) (M.≥ 40Y)

The age of child variables:

Less than 6 months ← **6 months to 1year** → **more than 1 year**

(Ch.< 6m) (Ch. 6m -1Y) (Ch.> 1Y)

The type of food variables:

Most of it protein ← **Most of it starch** → **Diverse**

(most protein) (most starch) (div.)

(Fig.3.1); scheme of treatments of the Experiments

*The variables has been classified according to their health effects ;(as in review of world Health Organization .

Chapter Four
Results &discussion

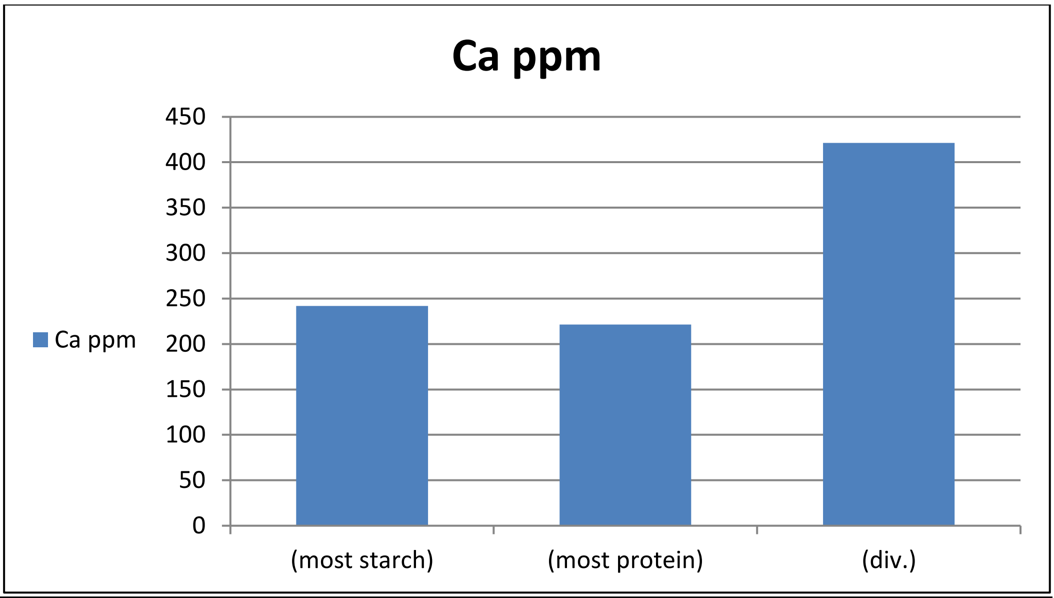
4.1- Experiment 1:

In this experiment we distributed questionnaire among mothers whom their age chosen to be less than 30 years as well as their children age less than 6 month. The food they ate is also taken into consideration as a function of age. Data in table(4.1) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

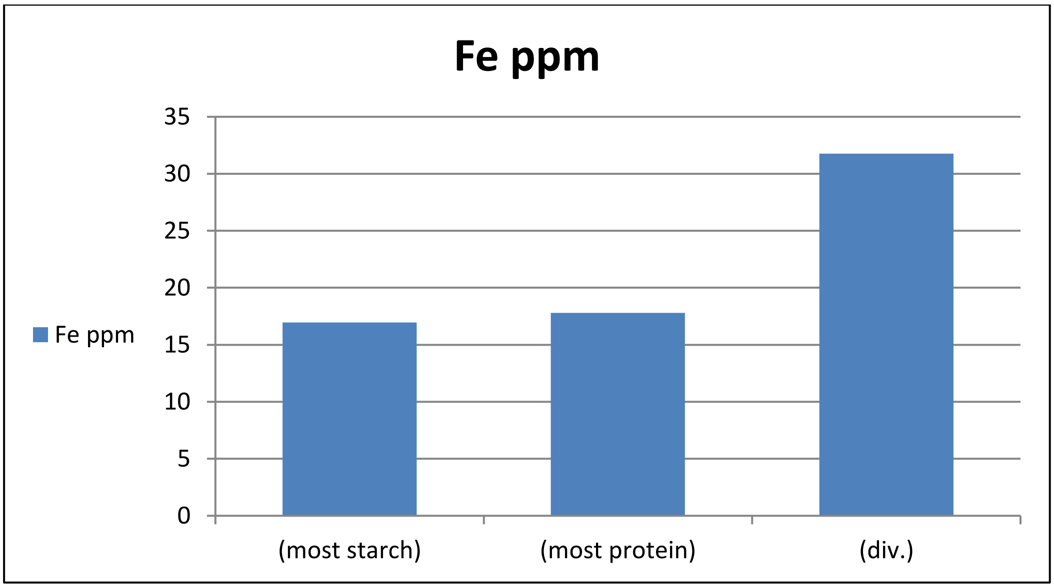
The relationship between each metal and the feeding system was viewed in table(4.1), fig.(4.1), (4.2), (4.3),and (4.4).

Table (4.1):the effect of food type on (ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age less than 30 year and their children less than 6 months old. (M.< 30Y * Ch.< 6m).

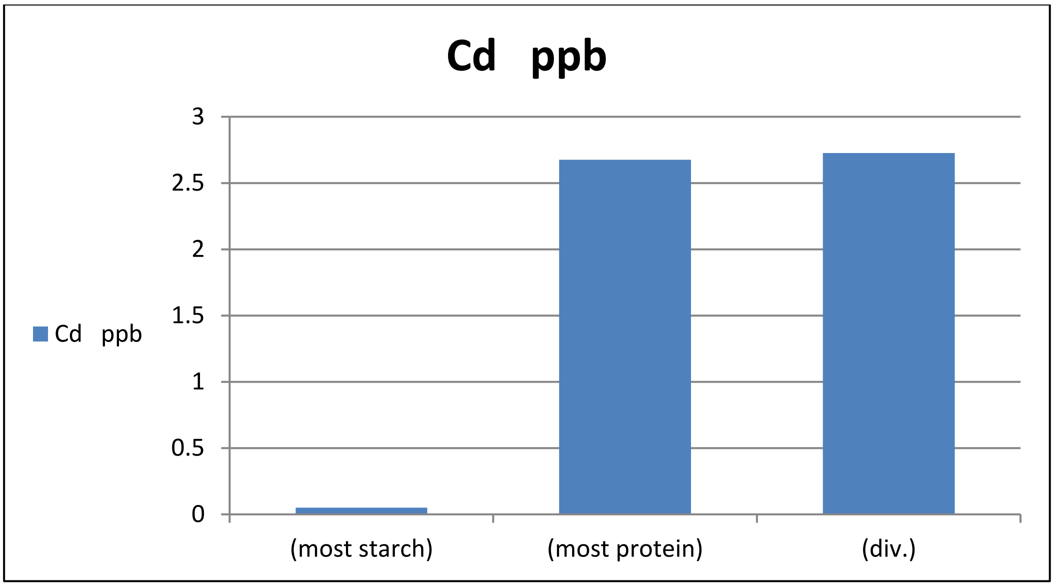
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca ppm	241.7710	221.5542	421.1955
Fe ppm	13.9599	17.7976	31.7542
Cd ppb	0.0495	2.6763	2.7273
Pb ppm	0.39575	0.2184	1.41535



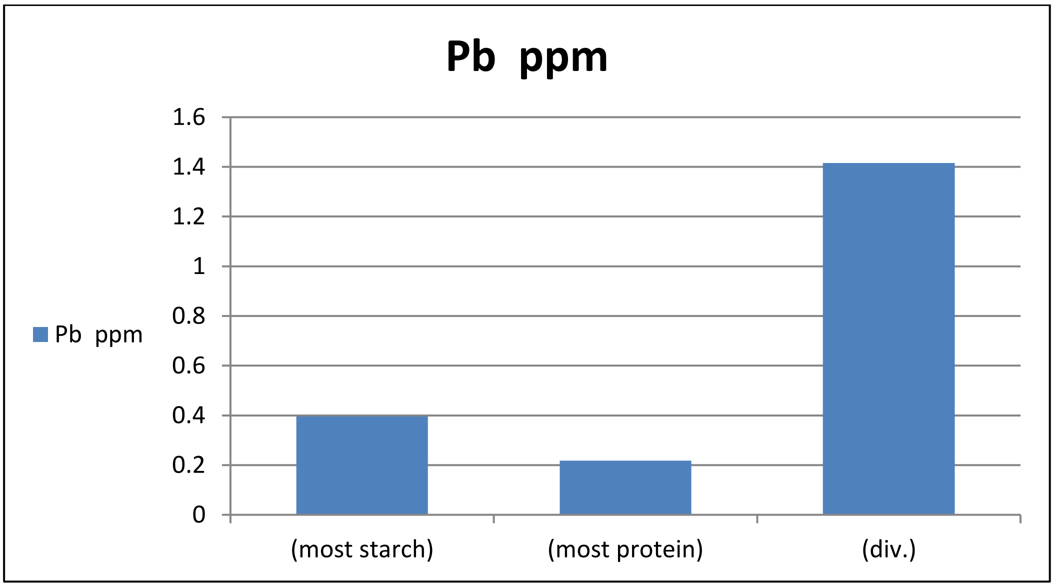
Fig;(4.1): The effect of variables of food type on the Ca ion concentration in mother milk whom their age less than 30 year and their children less than 6 months old. (M.< 30Y * Ch.< 6m).



Fig;(4.2): The effect of variables of food type on the Fe ion concentration in mother milk whom their age less than 30 year and their children less than 6 months old. (M.< 30Y * Ch.< 6m).



Fig;(4.3): The effect of variables of food type on the Cd ion concentration in mother milk whom their age less than 30 year and their children less than 6 months old. (M.< 30Y * Ch.< 6m).



Fig;(4.4): The effect of variables of food type on the Fe ion concentration in mother milk whom their age less than 30 year and their children less than 6 months old. (M.< 30Y * Ch.< 6m)..

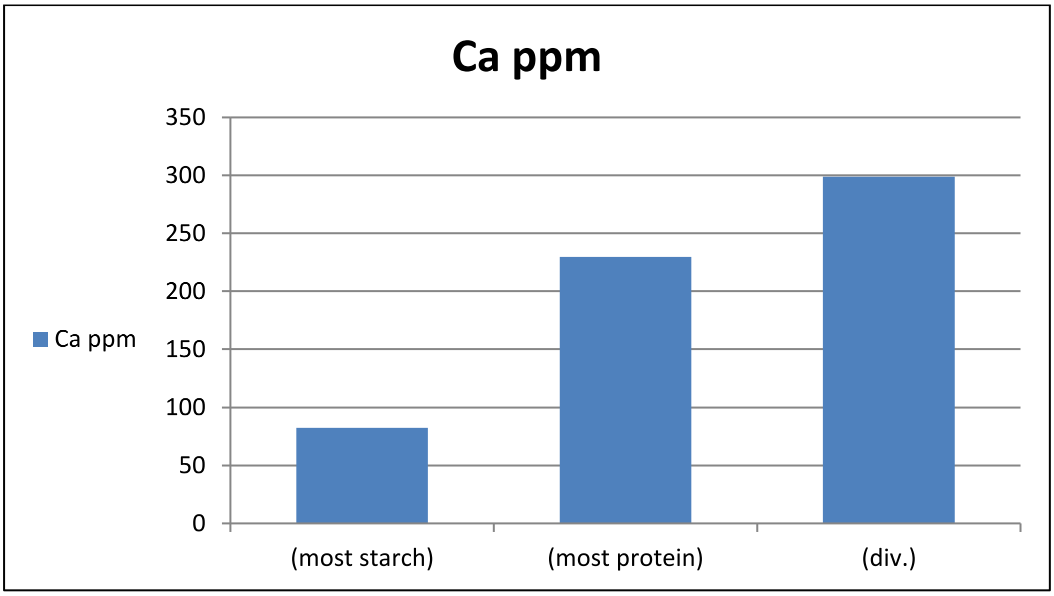
4.2- Experiment 2:

In this experiment we distributed questionnaire among mothers whom their age chosen to be less than 30 years as well as their children age between 6 month and 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.2) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

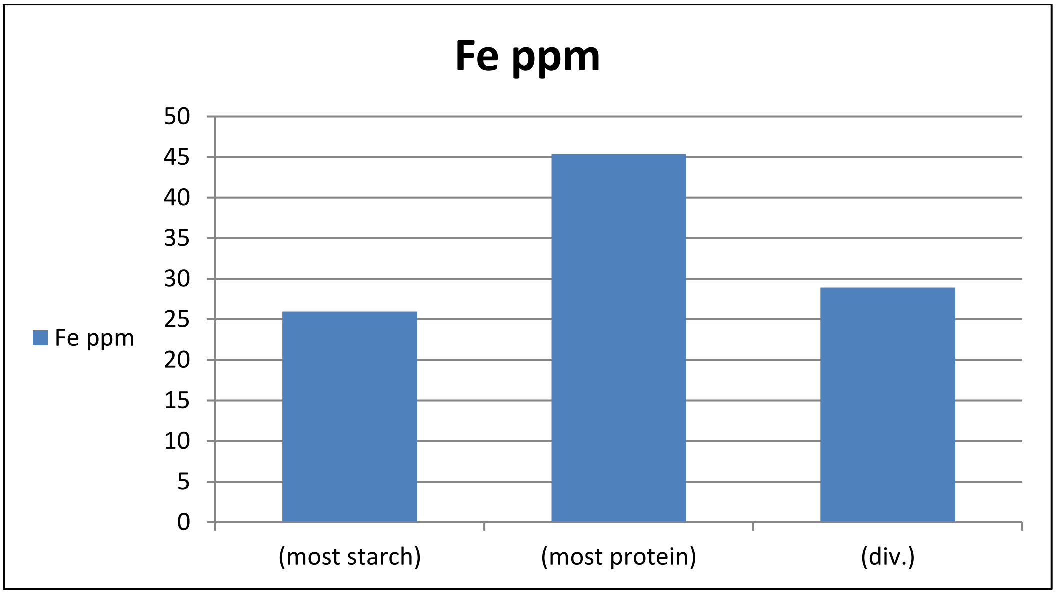
The relationship between each metal and the feeding system was viewed in fig.(4.5), (4.6), (4.7),and (4.8) .

Table (4.2):the effect of food type on (ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.) in mother milk whom their age less than 30 year and their children age between 6 months and 1year; (M.< 30Y * Ch. 6m-1y).

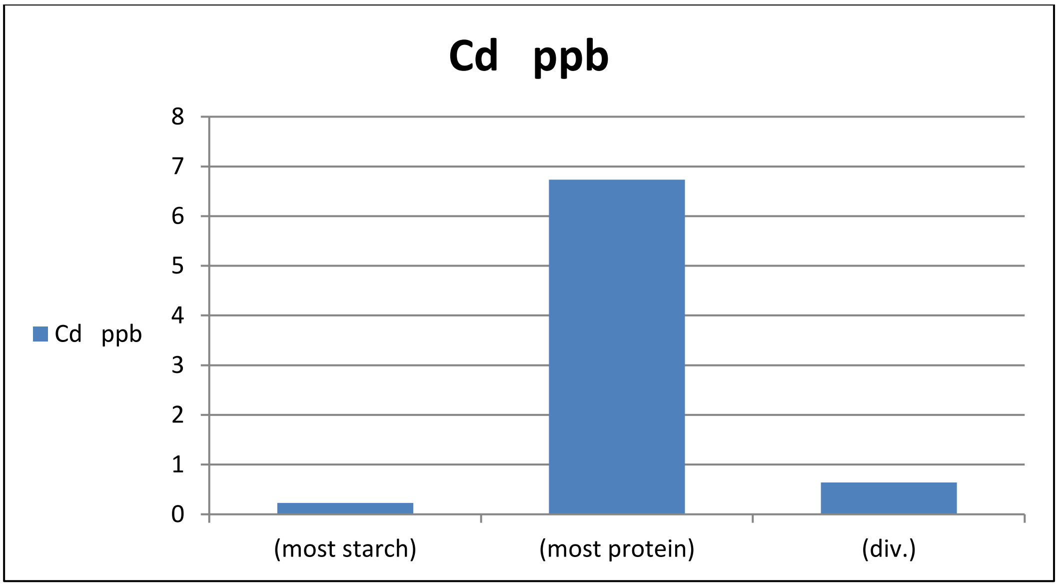
independent V. / **dependent V.**	**(most starch)**	**(most protein)**	**(div.)**
Ca	82.4973 ppm	230.0482 ppm	298.8108 ppm
Fe	25.9585 ppm	45.36315 ppm	28.9169 ppm
Cd	0.2306 ppb	6.73675 ppb	0.6414 ppb
Pb	0.5871 ppm	29.25185 ppm	0.91713 ppm



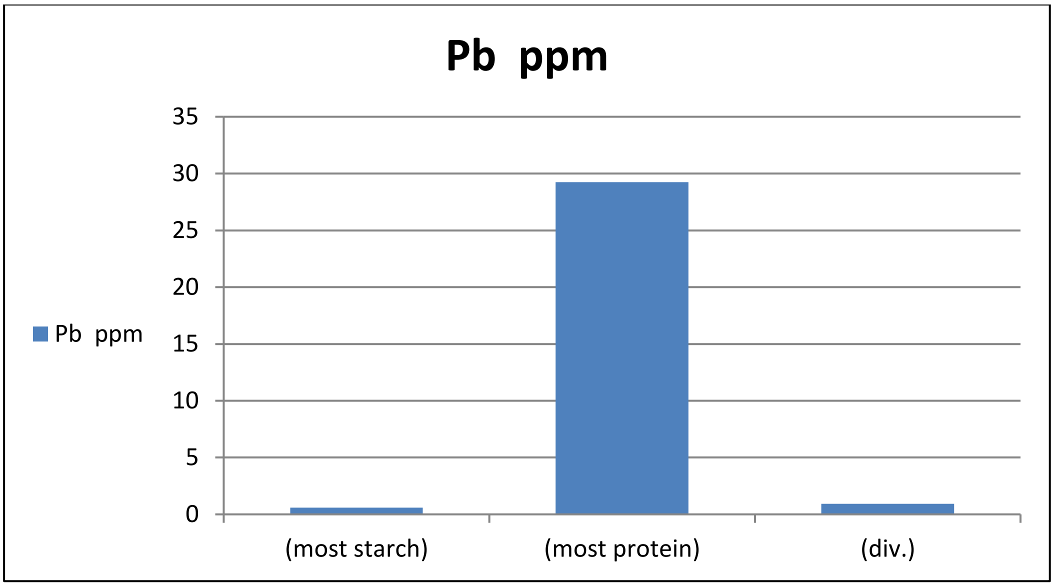
Fig;(4.5): The effect of variables of food type on the Ca ion concentration in mother milk whom their age less than 30 year and their children age between 6 months and 1year; (M.< 30Y * Ch. 6m-1y).



Fig;(4.6): The effect of variables of food type on the Fe ion concentration in mother milk whom their age less than 30 year and their children age between 6 months and 1year; (M.< 30Y * Ch. 6m-1y).



Fig;(4.7): The effect of variables of food type on the Cd ion concentration in mother milk whom their age less than 30 year and their children age between 6 months and 1year; (M.< 30Y * Ch. 6m-1y).



Fig;(4.8): The effect of variables of food type on the Pb ion concentration in mother milk whom their age less than 30 year and their children age between 6 months and 1year; (M.< 30Y * Ch. 6m-1y).

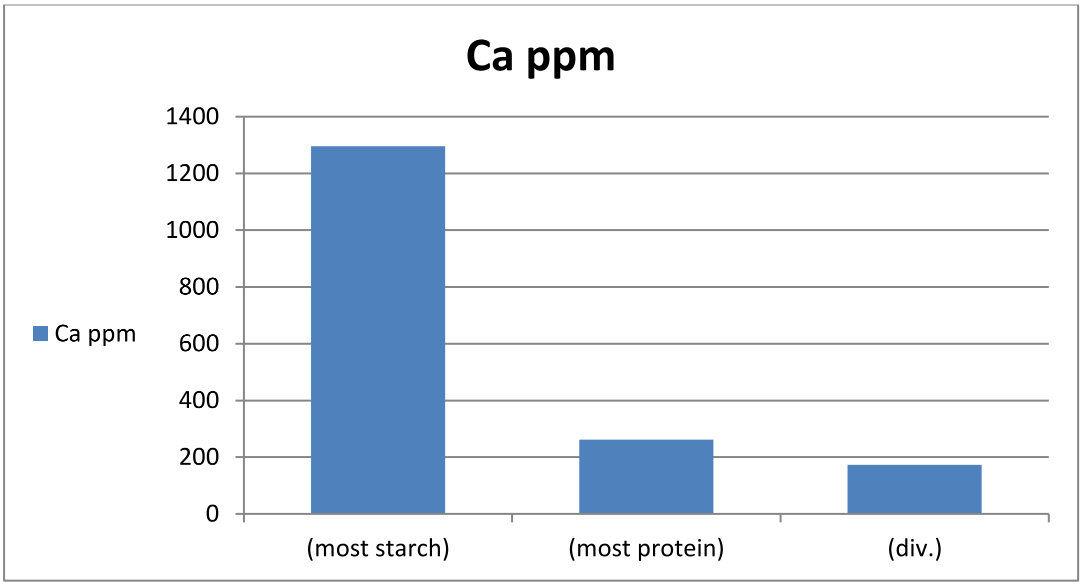
4.3- Experiment 3:

In this experiment we distributed questionnaire among mothers whom their age chosen to be less than 30 years as well as their children age more than 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.3) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

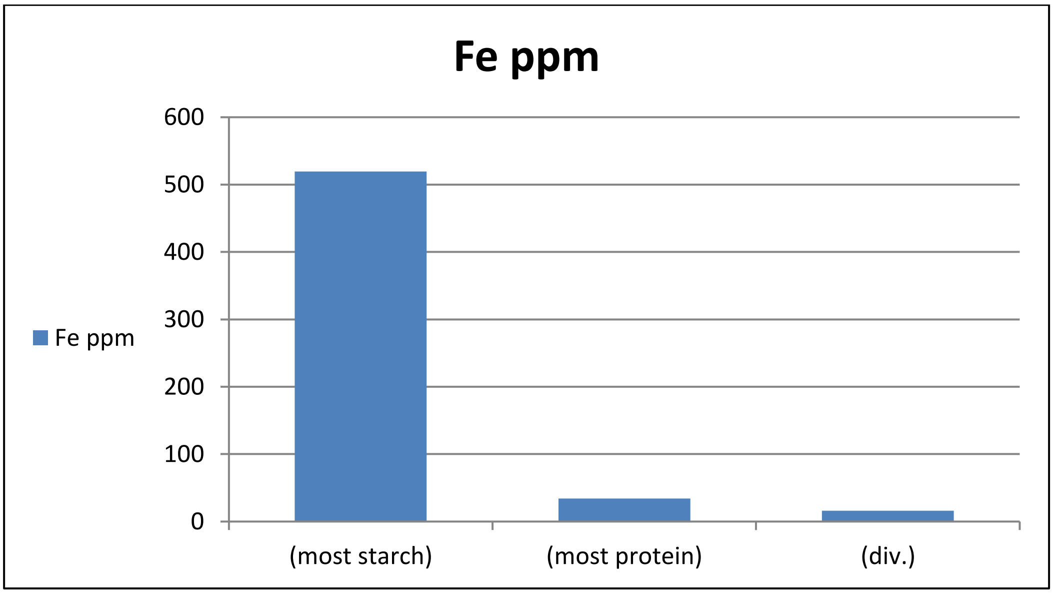
The relationship between each metal and the feeding system was viewed in fig.(4.9), (4.10), (4.11),and (4.12) .

Table (4.3):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age less than 30 year and their children age more than 1year;(M.< 30Y * Ch.>1Y).

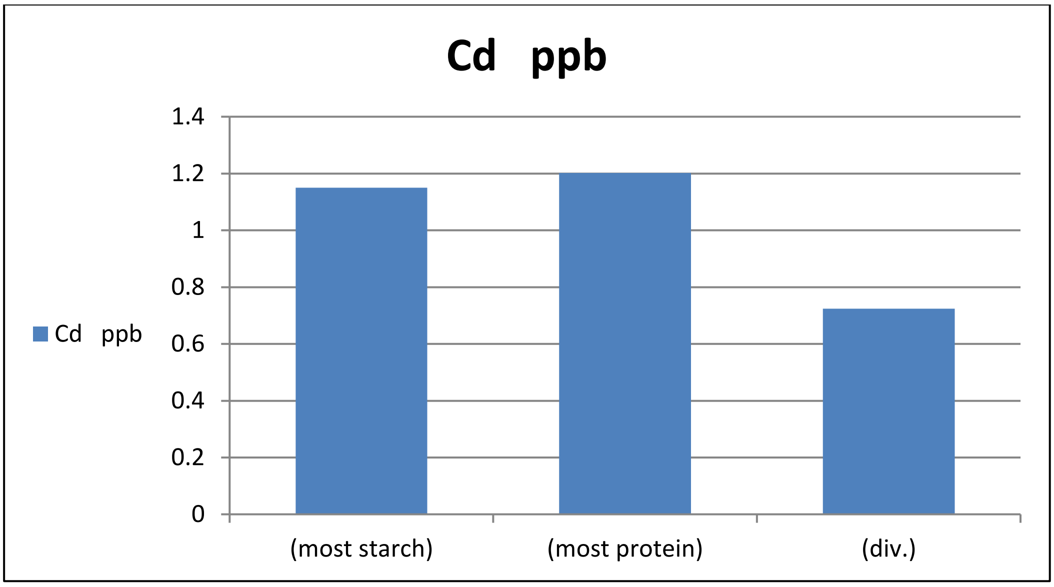
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	1295.1668 ppm	262.3521 ppm	173.2209 ppm
Fe	519.3722 ppm	34.2852 ppm	16.1900 ppm
Cd	1.1501 ppb	1.20215 ppb	0.7242 ppb
Pb	1.6899 ppm	1.4025 ppm	1.0227 ppm



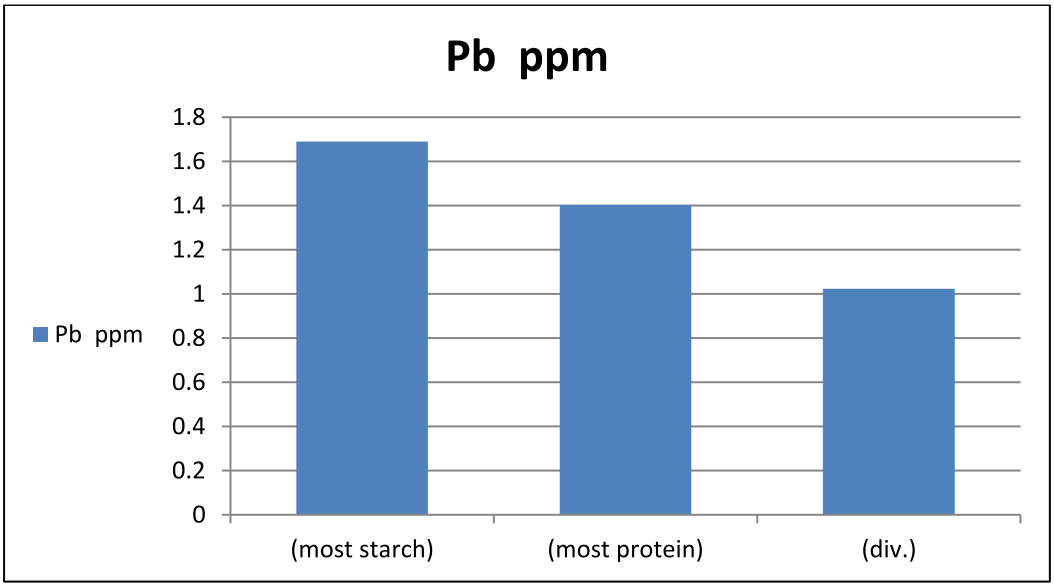
Fig;(4.9): The effect of variables of food type on the Ca ion concentration in mother milk whom their age less than 30 year and their children age more than 1year; (M.< 30Y * Ch.>1Y).



Fig;(4.10): The effect of variables of food type on the Fe ion concentration in mother milk whom their age less than 30 year and their children age more than 1year; (M.< 30Y * Ch.>1Y).



Fig;(4.11): The effect of variables of food type on the Cd ion concentration in mother milk whom their age less than 30 year and their children age more than 1year; (M.< 30Y * Ch.>1Y).



Fig;(4.12): The effect of variables of food type on the Pb ion concentration in mother milk whom their age less than 30 year and their children age more than 1year; (M.< 30Y * Ch.>1Y).

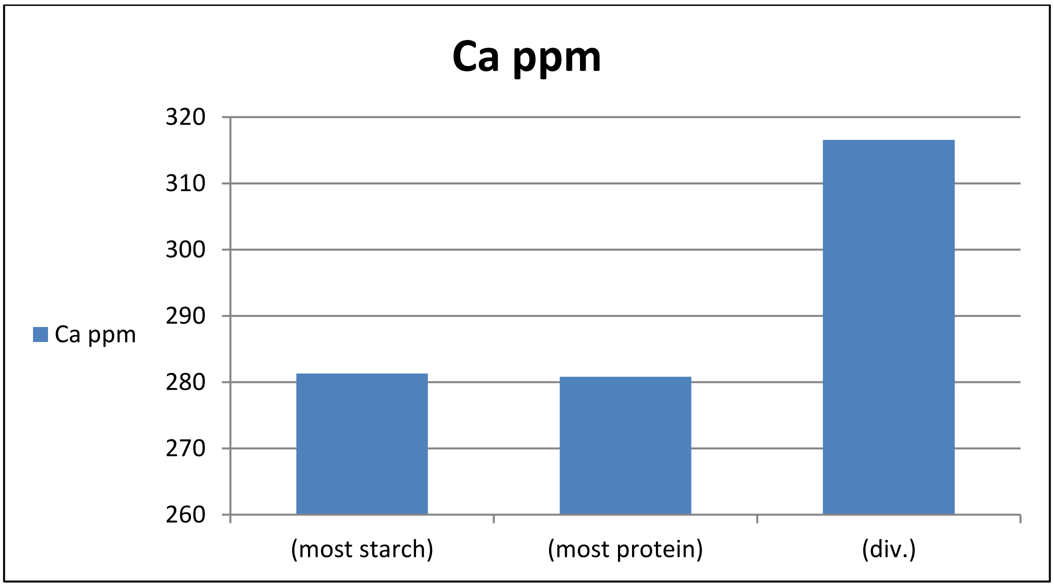
4.4- Experiment 4:

In this experiment we distributed questionnaire among mothers whom their age chosen to be between 30 and 39 years as well as their children age less than 6 month. The food they ate is also taken into consideration as a function of age. Data in table(4.4) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.   .

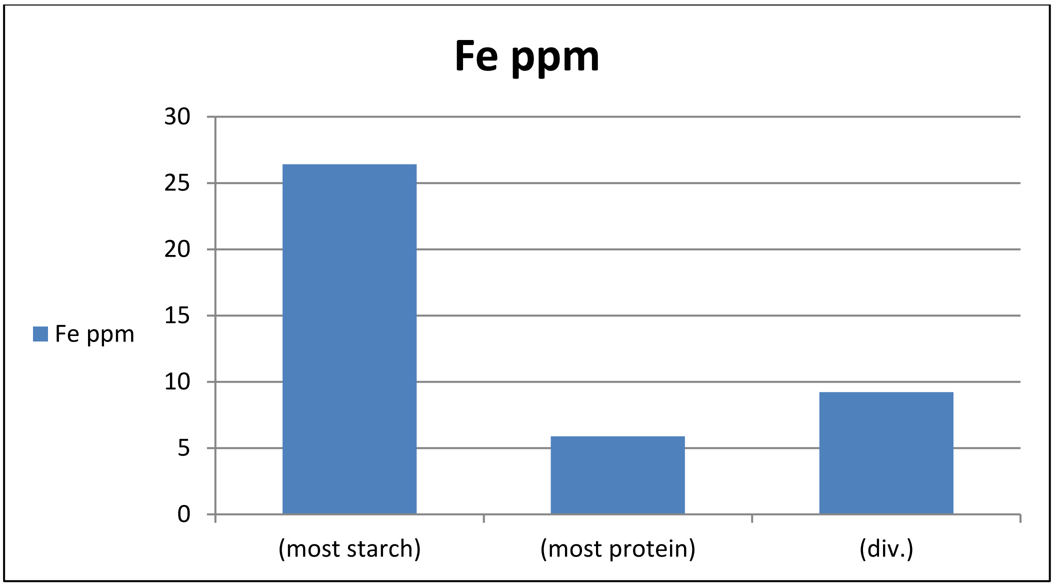
The relationship between each metal and the feeding system was viewed in fig.(4.13), (4.14), (4.15),and (4.16) .

Table (4.4):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age between 30 and 39 year and their children age less than 6 month; (M.30-39Y*Ch.<6m).

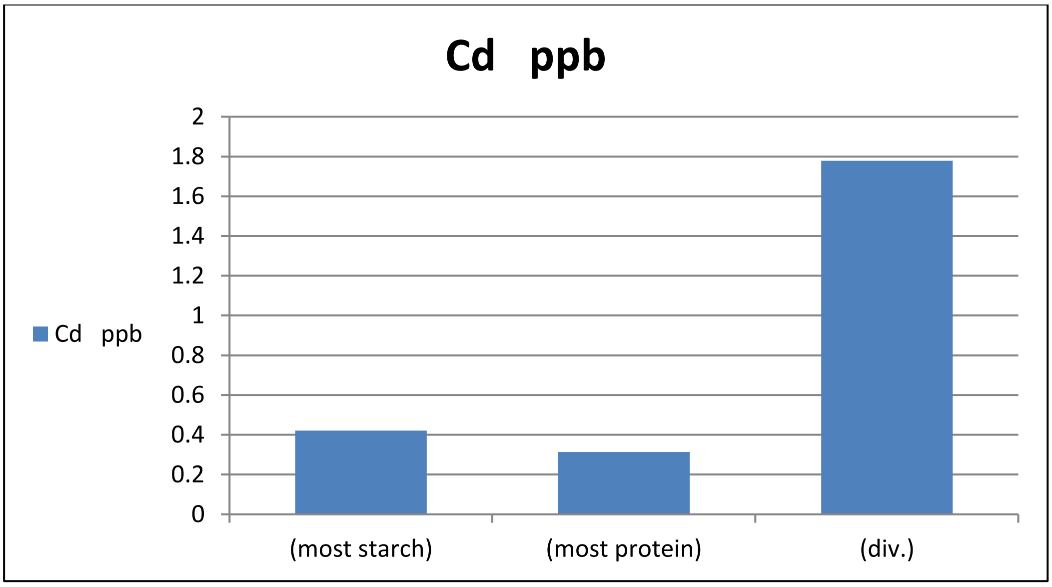
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	281.2958 **ppm**	280.8089 **ppm**	316.5426 **ppm**
Fe	26.4104 **ppm**	5.87932 **ppm**	9.2284 **ppm**
Cd	0.42125 **ppb**	0.31392 **ppb**	1.7788 **ppb**
Pb	0.73326 **ppm**	0.81378 **ppm**	2.0191 **ppm**



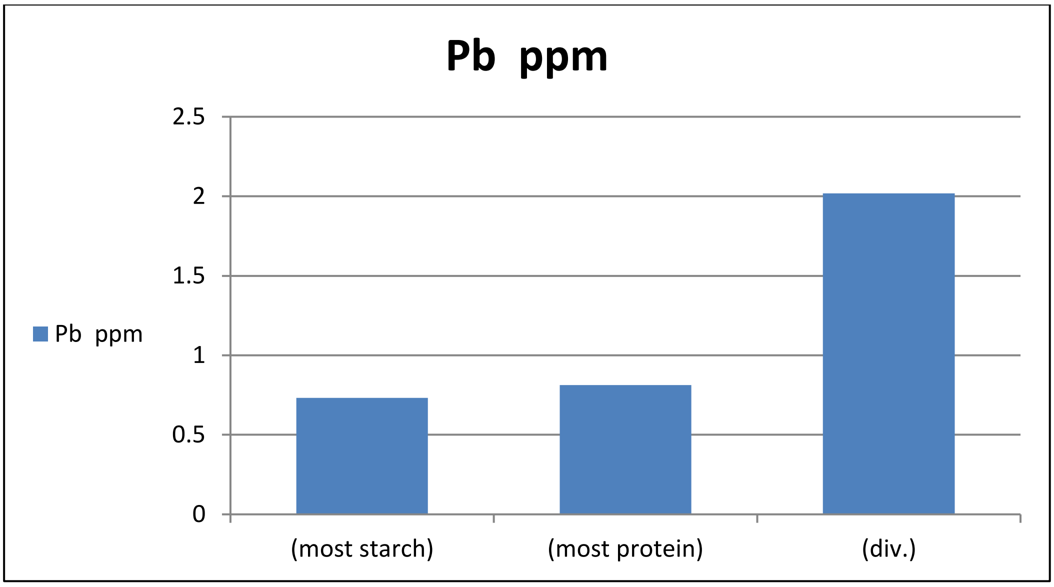
Fig;(4.13): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age between 30 and 39 year and their children age less than 6 month; (M.30-39Y*Ch.<6m).



Fig;(4.14): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age between 30 and 39 year and their children age less than 6 month; (M.30-39Y*Ch.<6m).



Fig;(4.15): The effect of variables of type of food on the Cd ion concentration in mother milk whom their age between 30 and 39 year and their children age less than 6 month; (M.30-39Y*Ch.<6m).



Fig;(4.16): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age between 30 and 39 year and their children age less than 6 month; (M.30-39Y*Ch.<6m).

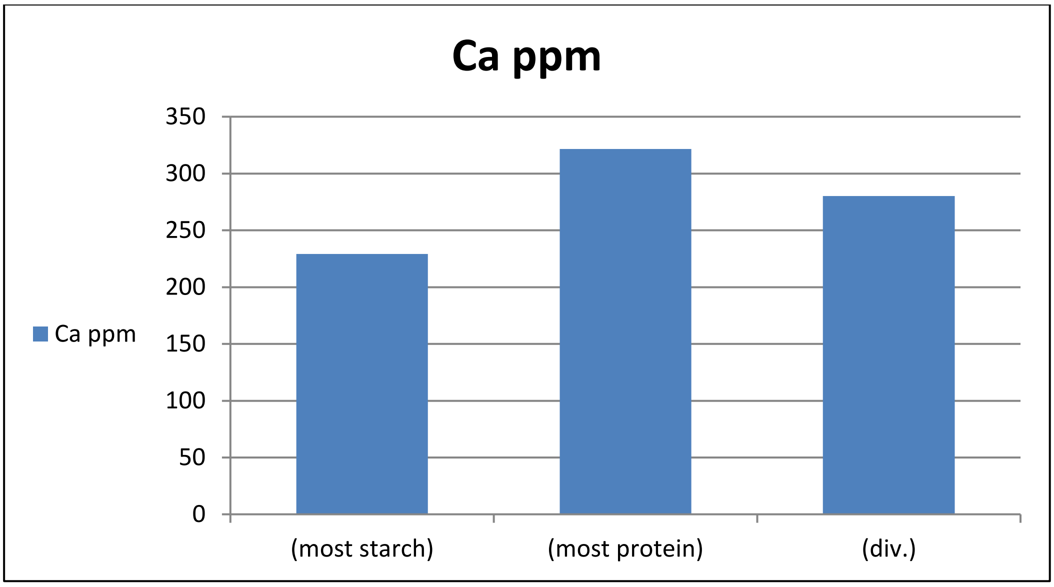
4.5- Experiment 5:

In this experiment we distributed questionnaire among mothers whom their age chosen to be between 30 and 39 years as well as their children age between 6 month and 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.5) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

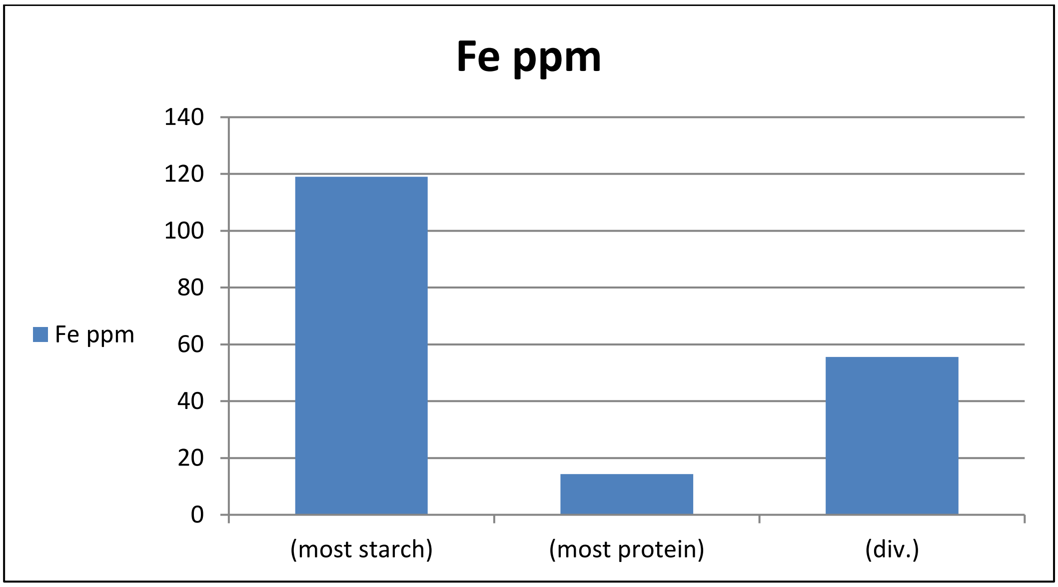
The relationship between each metal and the feeding system was viewed in fig.(4.17), (4.18), (4.19),and (4.20).

Table (4.5):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age between 30 and 39 year and their children age between 6 month and 1 year; (M.30- 39Y *Ch. 6m-1y).

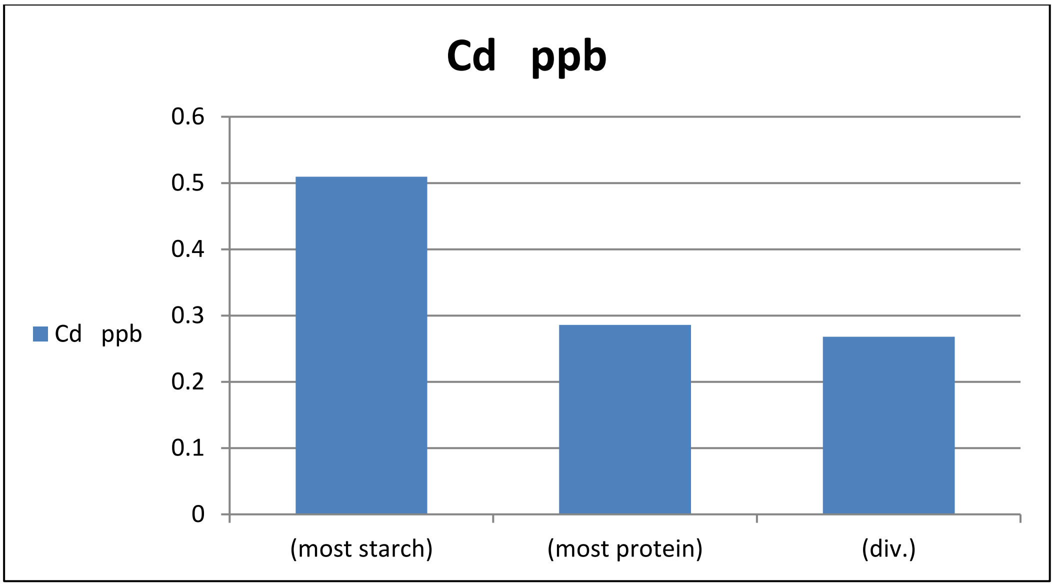
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	229.1980 ppm	321.7310 ppm	280.1276 ppm
Fe	119.0306 ppm	14.3819 ppm	55.544 ppm
Cd	0.50952 ppb	0.2861 ppb	0.2679 ppb
Pb	1.18967 ppm	0.92365 ppm	0.8046 ppm



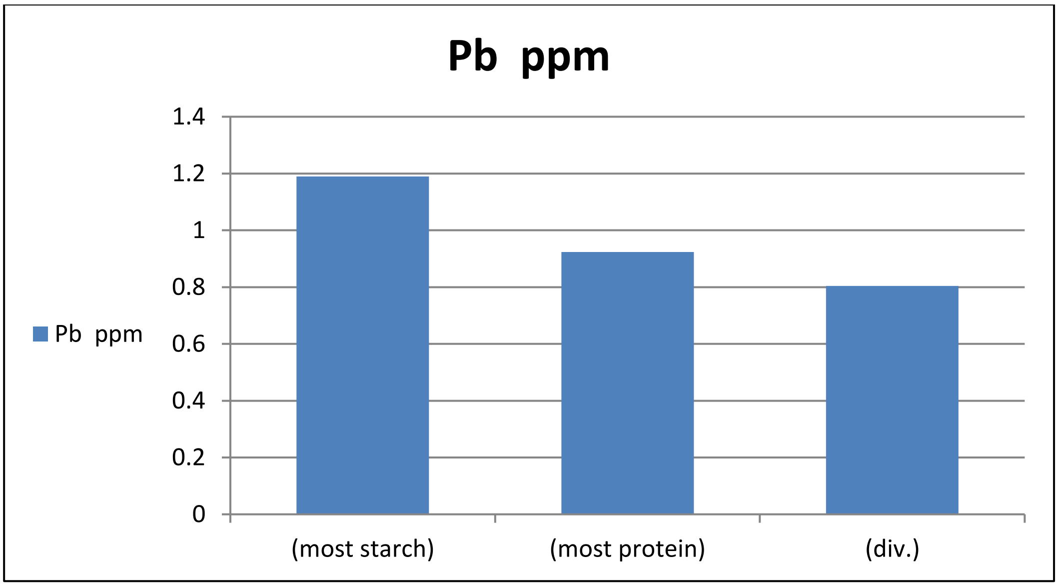
Fig;(4.17): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age between 30 and 39 year and their children age between 6 month and 1 year; (M.30- 39Y *Ch. 6m-1y).



Fig;(4.18): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age between 30 and 39 year and their children age between 6 month and 1 year; (M.30- 39Y *Ch. 6m-1y).



Fig;(4.19): The effect of variables of type of food on the Cd ion concentration in mother milk whom their age between 30 and 39 year and their children age between 6 month and 1 year; (M.30- 39Y *Ch. 6m-1y).



Fig;(4.20): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age between 30 and 39 year and their children age between 6 month and 1 year;(M.30- 39Y *Ch. 6m-1y).

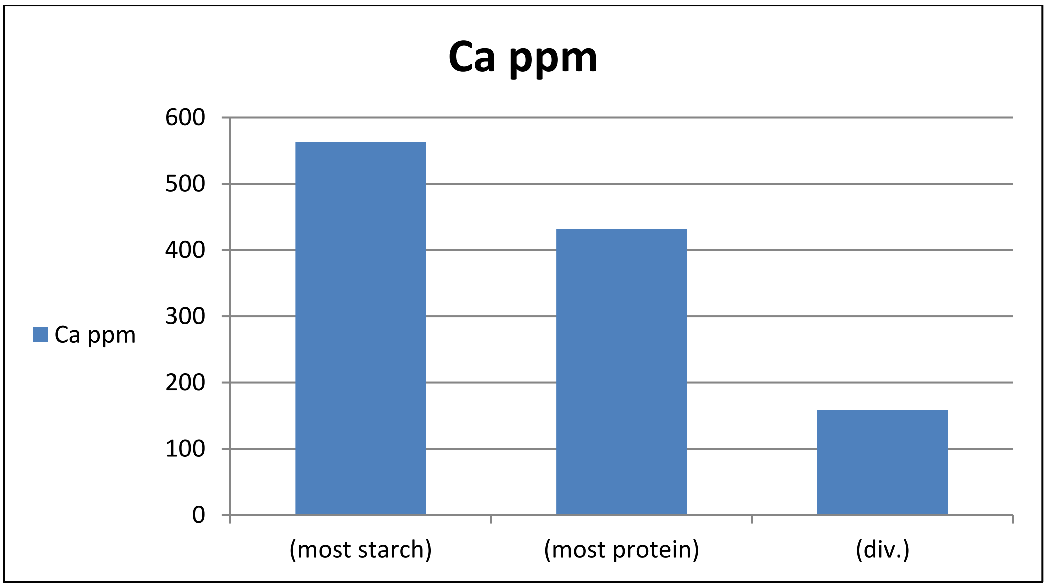
4.6- Experiment 6:

In this experiment we distributed questionnaire among mothers whom their age chosen to be between 30 and 39 years as well as their children age more than 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.6) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

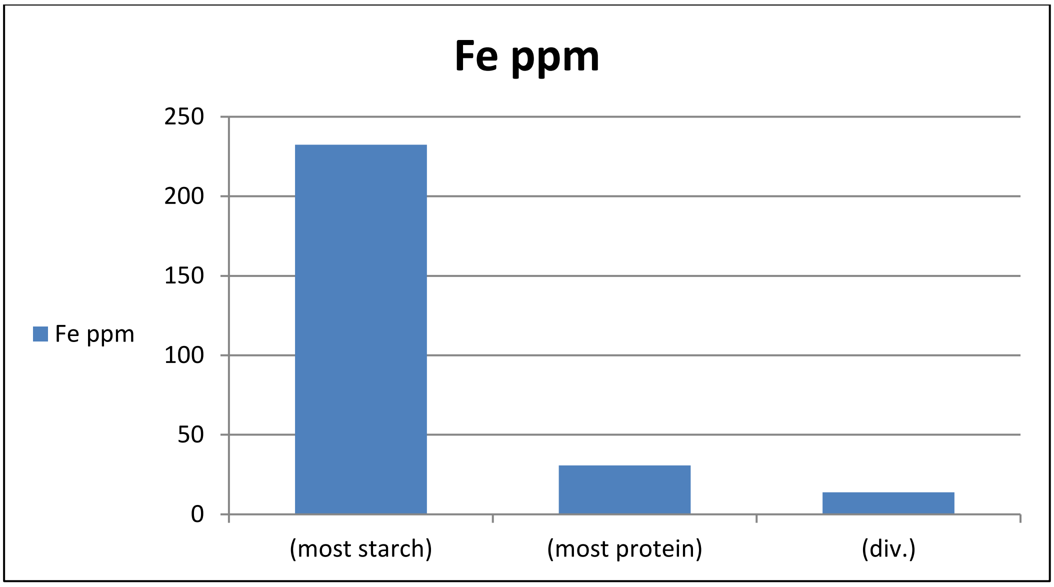
The relationship between each metal and the feeding system was viewed in fig.(4.21), (4.22), (4.23),and (4.24) .

Table (4.6):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age between 30 and 39 year and their children age more than 1 year;(M.30- 39Y * Ch.>1Y).

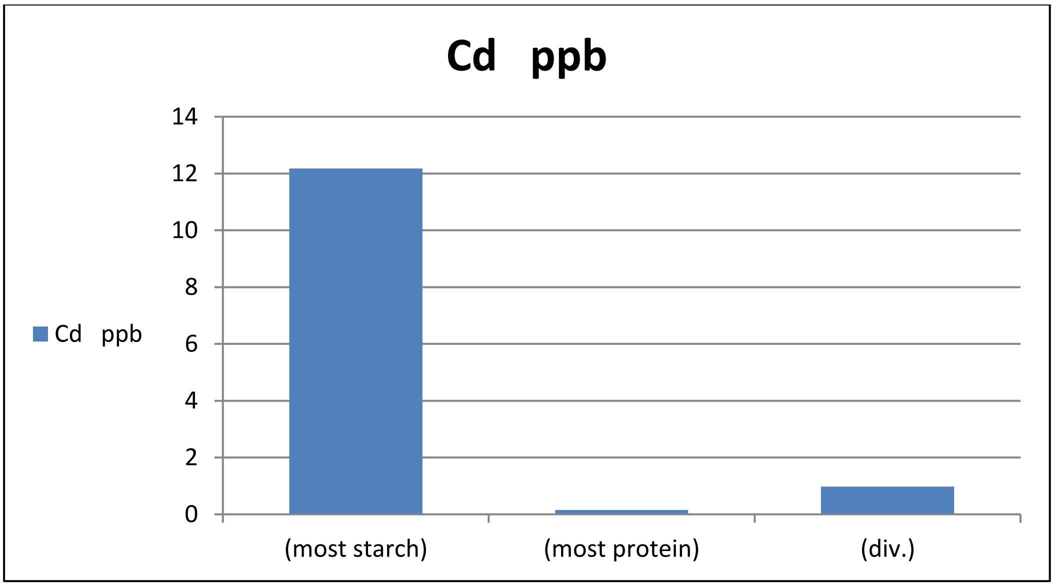
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	563.1720 ppm	432.0580 ppm	158.3010 ppm
Fe	232.3408 ppm	30.7747 ppm	13.9292 ppm
Cd	12.1795 ppb	0.1558 ppb	0.9757 ppb
Pb	1.5767 ppm	2.0072 ppm	1.4558 ppm



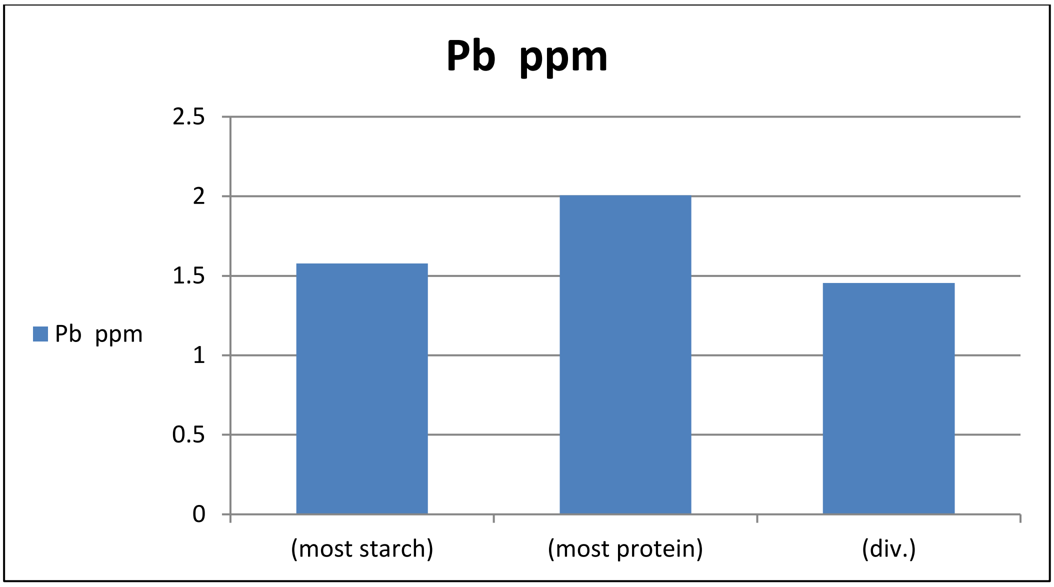
Fig;(4.21): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age between 30 and 39 year and their children age more than 1 year;(M.30- 39Y * Ch.>1Y).



Fig;(4.22): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age between 30 and 39 year and their children age more than 1 year;(M.30- 39Y * Ch.>1Y).



Fig;(4.23): The effect of variables of type of food on the Cd ion concentration in mother milk whom their age between 30 and 39 year and their children age more than 1 year;(M.30- 39Y * Ch.>1Y).



Fig;(4.24): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age between 30 and 39 year and their children age more than 1 year;(M.30- 39Y * Ch.>1Y).

4.7- Experiment 7:

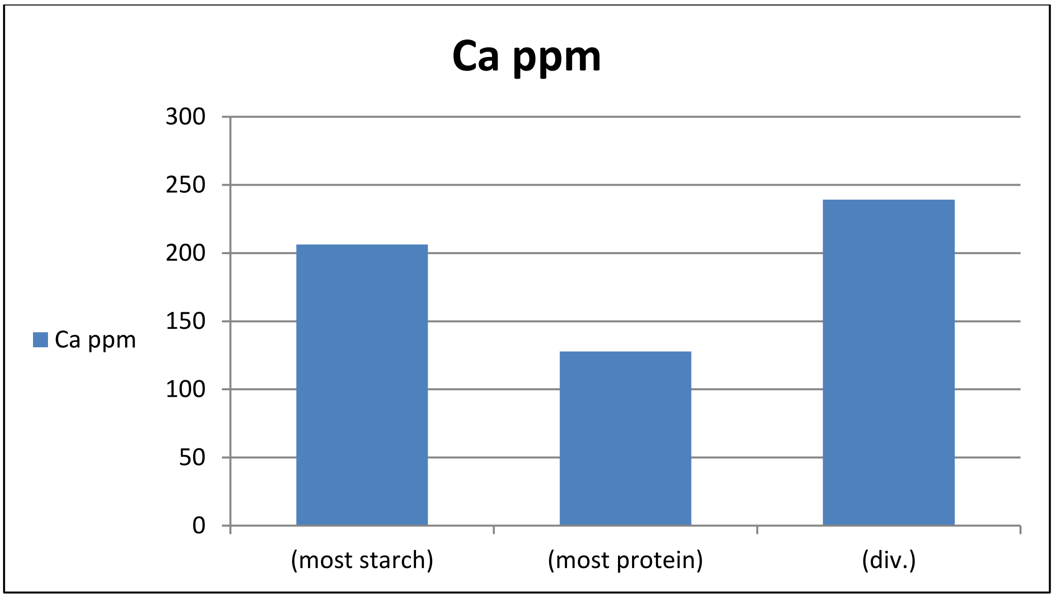
In this experiment we distributed questionnaire among mothers whom their age chosen to be 40 years and above as well as their children age less than 6 month. The food they ate is also taken into consideration as a function of age. Data in table(4.7) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

The relationship between each metal and the feeding system was viewed in fig.(4.25), (4.26), (4.27),and (4.28) .

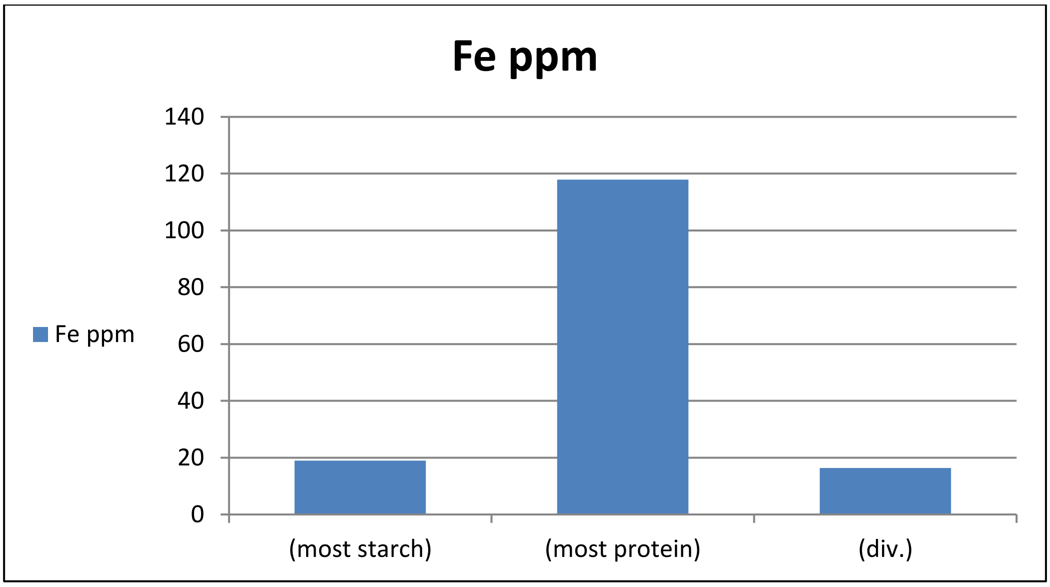
Table (4.7):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age 40 year and above, and their children age less than 6 month; (M.≥40* Ch.< 6m).

independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	206.3773 ppm	127.7236 ppm	239.3216 ppm
Fe	18.9945 ppm	117.8965 ppm	16.3736 ppm

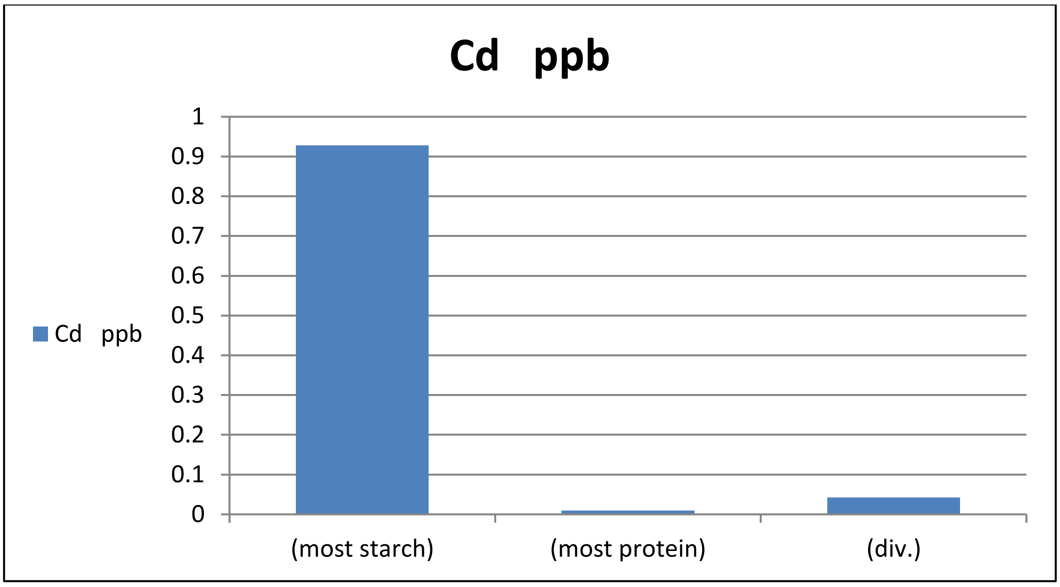
Cd	0.9284 ppb	0.0093 ppb	0.0423 ppb
Pb	1.07605 ppm	0.2091 ppm	0.41903 ppm



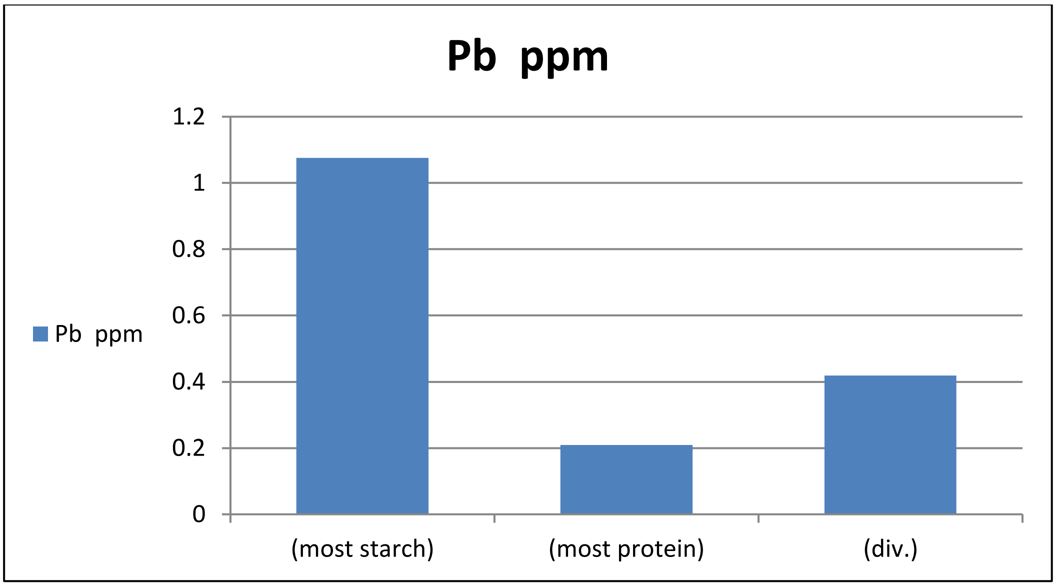
Fig;(4.25): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age 40 year and above, and their children age less than 6 month;(M.≥40* Ch.< 6m).



Fig;(4.26): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age 40 year and above, and their children age less than 6 month;(M.≥40* Ch.< 6m).



Fig;(4.27): The effect of variables of type of food on the Cd ion V in mother milk whom their age 40 year and above, and their children age less than 6 month;(M.≥40* Ch.< 6m).



Fig;(4.28): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age 40 year and above, and their children age less than 6 month;(M.≥40* Ch.< 6m).

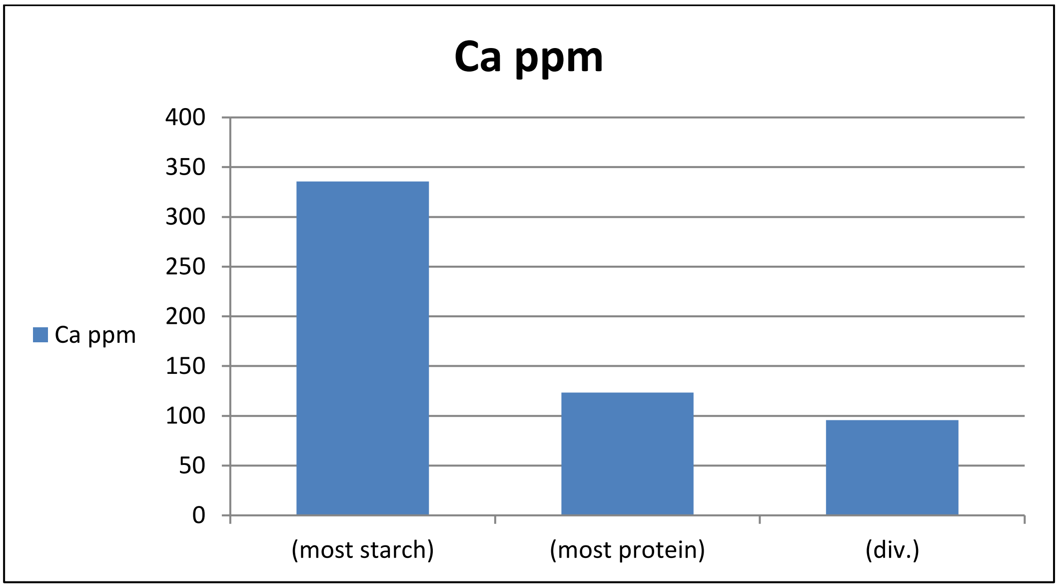
4.8- Experiment 8:

In this experiment we distributed questionnaire among mothers whom their age chosen to be 40 years and above as well as their children age between 6 month and 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.8) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

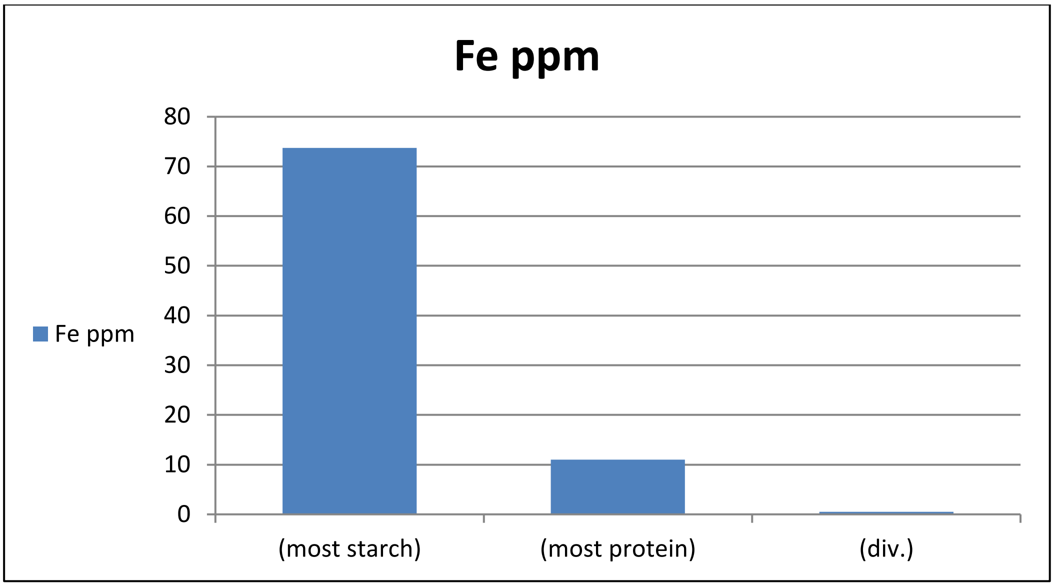
The relationship between each metal and the feeding system was viewed in fig.(4.29), (4.30), (4.31),and (4.32) .

Table (4.8):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age 40 year and above, and their children age between 6 month and 1 year ;(M.≥40* Ch.6m –1y).

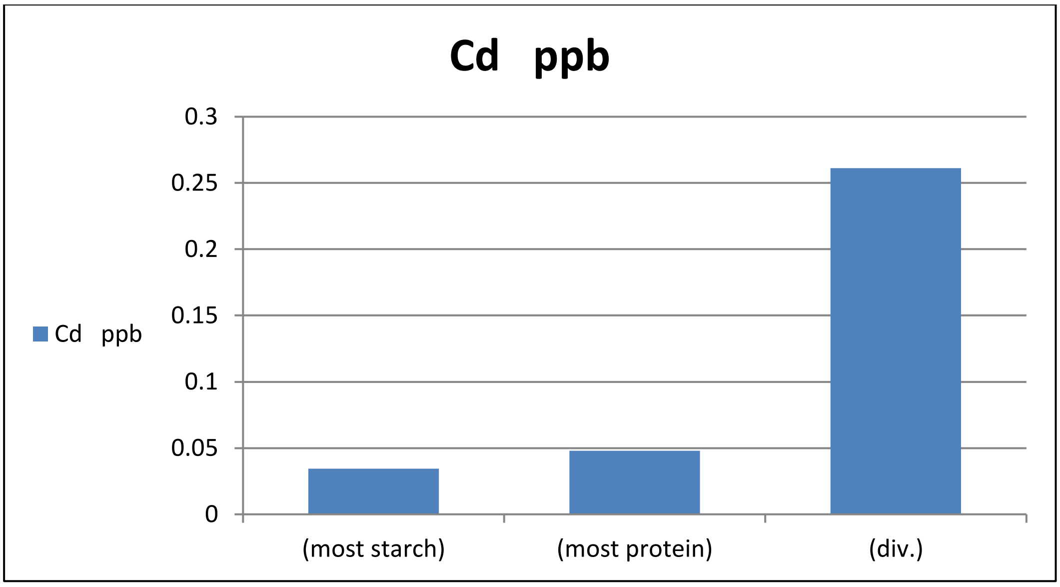
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	335.6212 ppm	123.4039 ppm	95.8927 ppm
Fe	73.7365 ppm	10.9795 ppm	0.4802 ppm
Cd	0.03443 ppb	0.04793 ppb	0.2611 ppb
Pb	0.58363 ppm	0.57387 ppm	0.4557 ppm



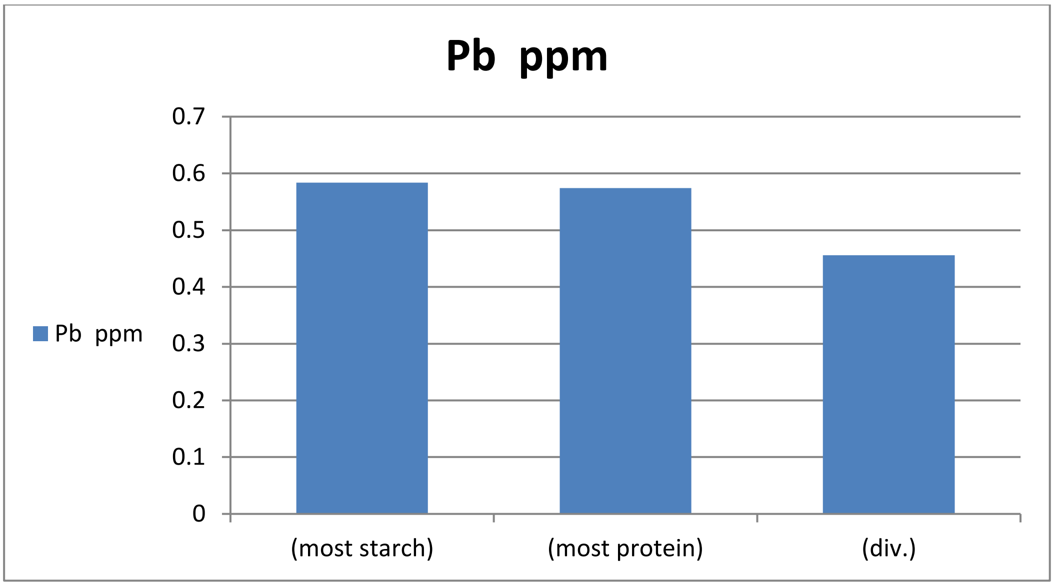
Fig;(4.29): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age 40 year and above, and their children age between 6 month and 1 year ;(M.≥40* Ch.6m –1y).



Fig;(4.30): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age 40 year and above, and their children age between 6 month and 1 year ;(M.≥40* Ch.6m –1y).



Fig;(4.31): The effect of variables of type of food on the Cd ion concentration in mother milk whom their age 40 year and above, and their children age between 6 month and 1 year ;(M.≥40* Ch.6m –1y).



Fig;(4.32): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age 40 year and above, and their children age between 6 month and 1 year;(M.≥40* Ch.6m –1y).

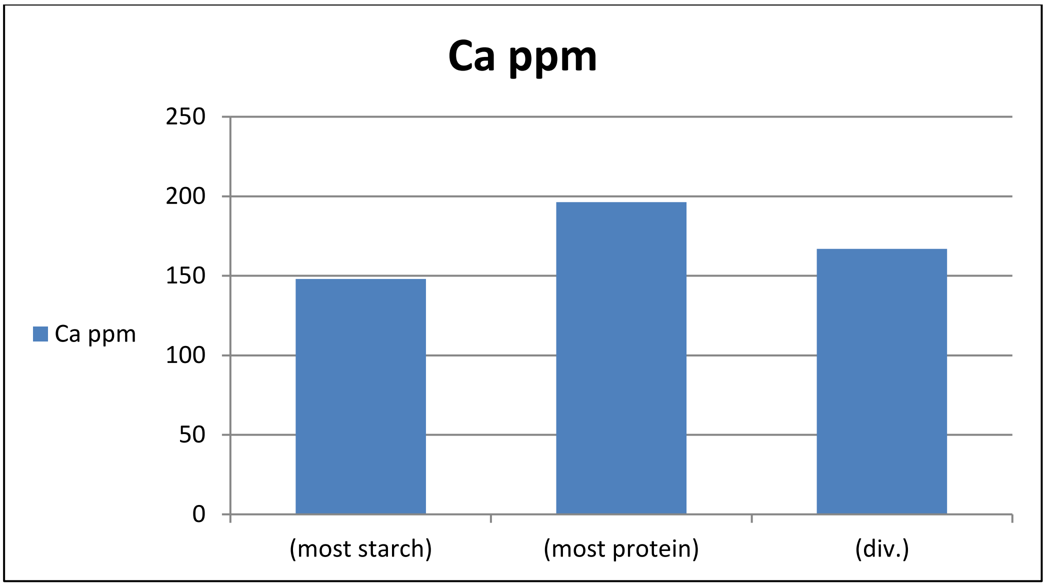
4.9- Experiment 9:

In this experiment we distributed questionnaire among mothers whom their age chosen to be 40 years and above as well as their children age more than 1 year. The food they ate is also taken into consideration as a function of age. Data in table(4.9) shows the variation of (Ca, Fe, Cd, and Pb) metals concentration in feeding milk as function of food type.

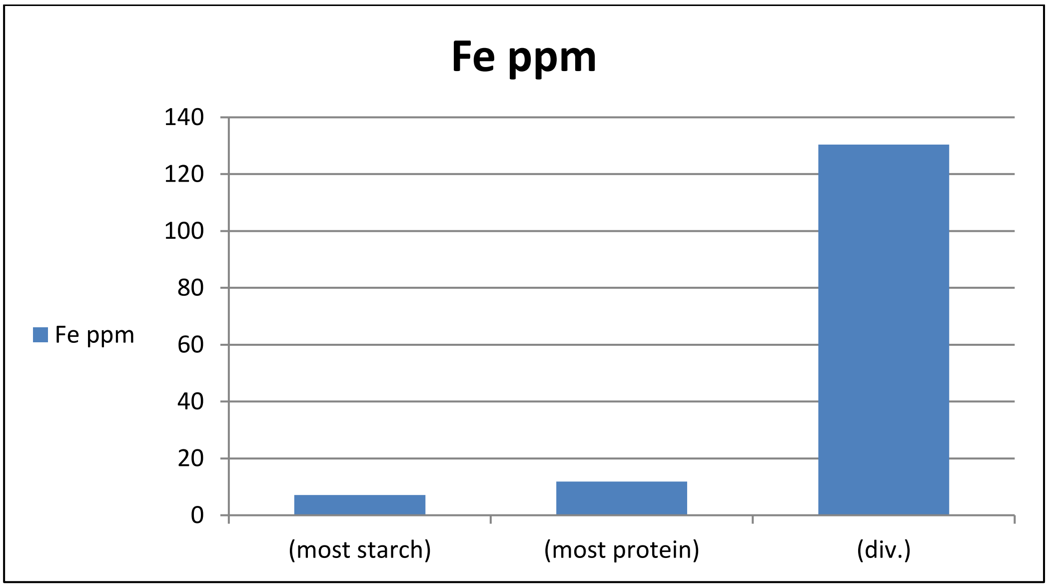
The relationship between each metal and the feeding system was viewed in fig.(4.33), (4.34), (4.35),and (4.36) .

Table (4.9):the effect of type of food on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.)in mother milk whom their age 40 year and above, and their children age more than 1 year ;(M.≥40* Ch.>1y).

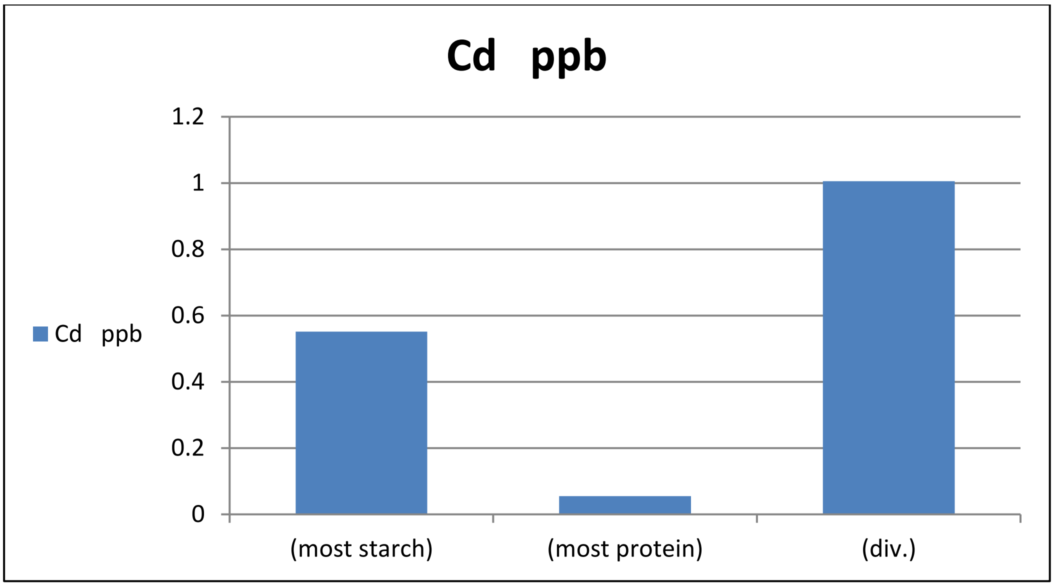
independent V. / dependent V.	(most starch)	(most protein)	(div.)
Ca	148.0189 ppm	196.3938 ppm	166.9021 ppm
Fe	7.0903 ppm	11.8801 ppm	130.4522 ppm
Cd	0.5517 ppb	0.0547 ppb	1.0051 ppb
Pb	1.17395 ppm	0.4672 ppm	1.9128 ppm



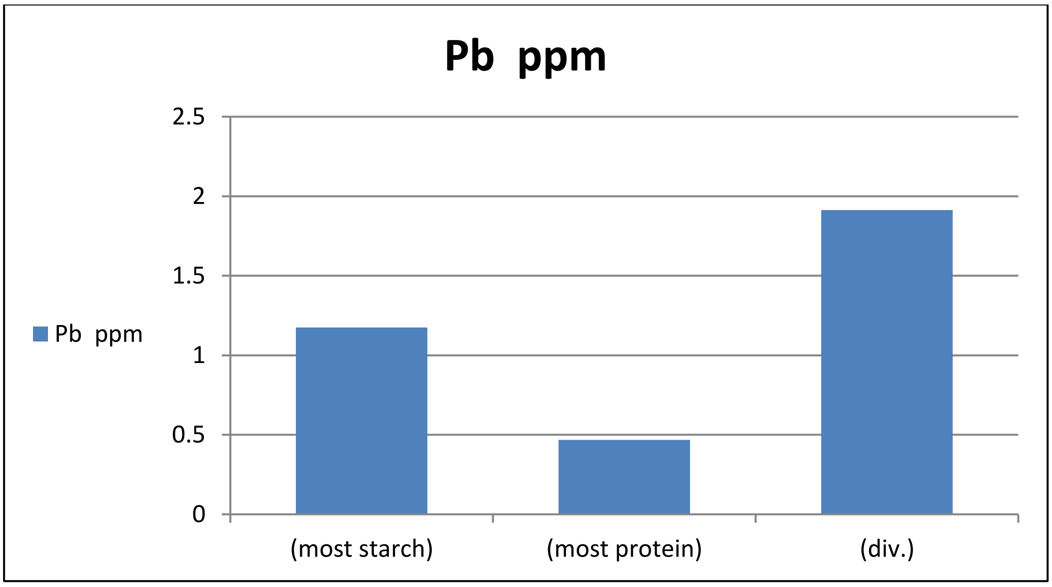
Fig;(4.33): The effect of variables of type of food on the Ca ion concentration in mother milk whom their age 40 year and above, and their children age more than 1 year ;(M.≥40* Ch.>1y).



Fig;(4.34): The effect of variables of type of food on the Fe ion concentration in mother milk whom their age 40 year and above, and their children age more than 1 year ;(M.≥40* Ch.>1y).



Fig;(4.35): The effect of variables of type of food on the Cd ion concentration in mother milk whom their age 40 year and above, and their children age more than 1 year ;(M.≥40* Ch.>1y).



Fig;(4.36): The effect of variables of type of food on the Pb ion concentration in mother milk whom their age 40 year and above, and their children age more than 1 year ;(M.≥40* Ch.>1y).

4.10- Experiment 10:

In this experiment we collected a many samples from each three types of source of milk; **(** Cows , Industrial's, and Human Mothers **)** which is so called independent variables in this experiment**,** and then we determined the values of;(Ca, Fe, Cd, and Pb) metals;- which represents the dependent variables- in all samples of milk, by using Atomic Absorption spectrophotometer .

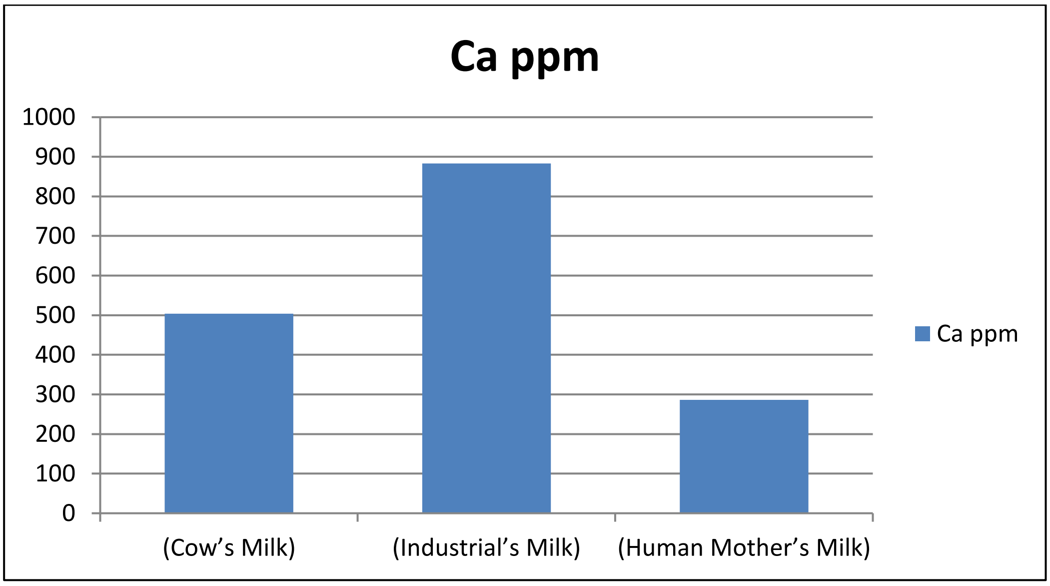
The average of each above values has been calculated according to the type of milk's sources.

The values of the metals was viewed in table (4.10) to show the relationship between the present of these (Ca, Fe, Cd, and Pb) metals and the variables of sources;(if it is Cows , Industrial's, or Human Mothers) .

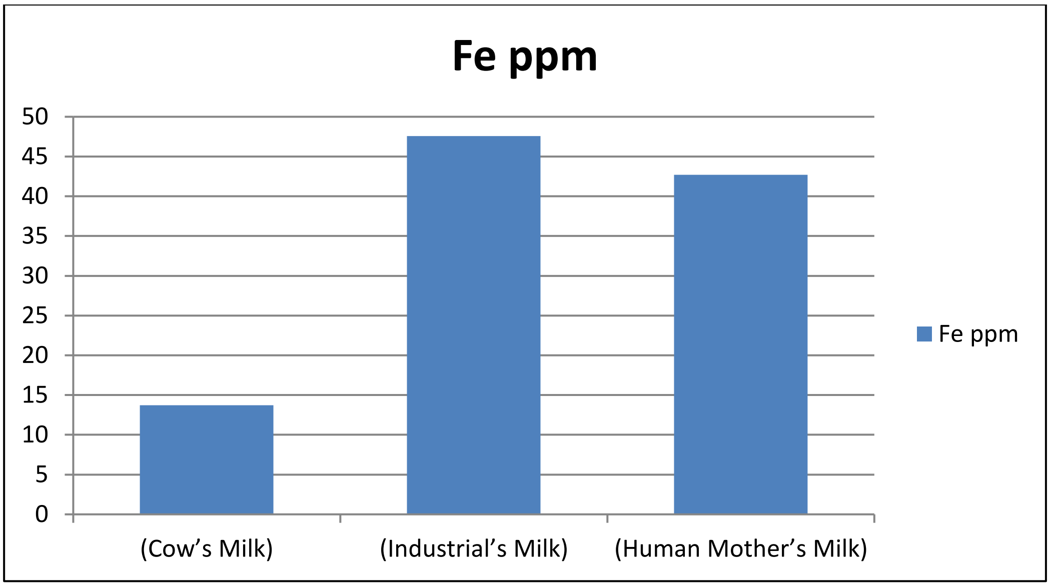
The relationship between each metal concentration and the source of milk was viewed in fig.(4.37), (4.38), (4.39),and (4.40) .

Table (4.10):the effect of types of source of milk if it's; (Cows , Industrial's, or Human Mothers)on(ca, Fe, Cd, and Pb) metals concentration variables (M. C.V.):

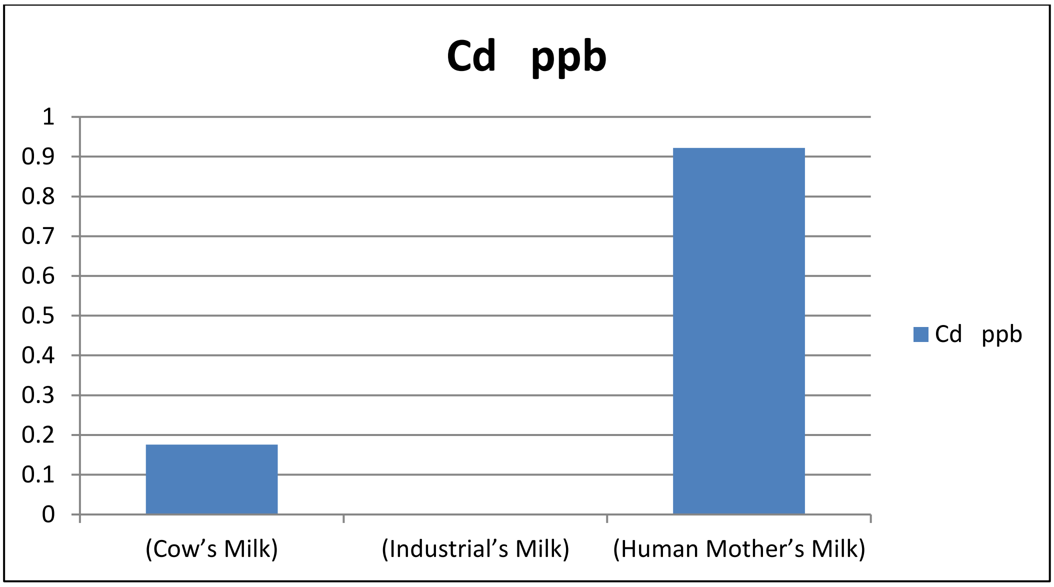
independent V. / dependent V.	(Cow's Milk)	(Industrial's Milk)	(Human Mother's Milk)
Ca	503.4629 ppm	883.1408 ppm	286.2776 ppm
Fe	13.7101 ppm	47.5766 ppm	42.6850 ppm
Cd	0.1761 ppb	o.o442 ppb	0.9216 ppb
Pb	0.5721 ppm	0.51438 ppm	1.0025 ppm



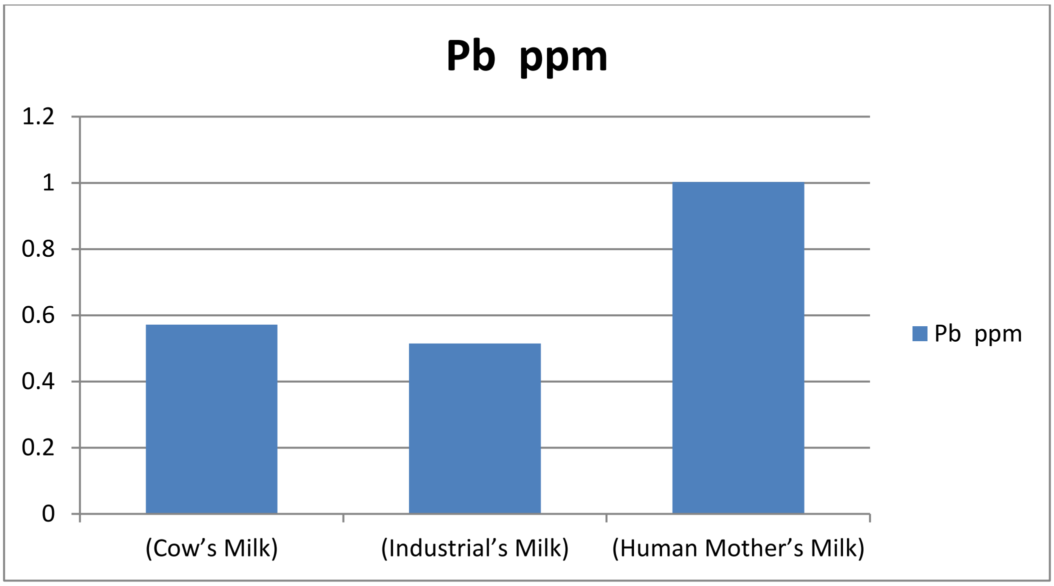
Fig;(4.37): The effect of variables of source;(Cows , Industrial's, and Human Mothers) on the Ca ion concentration in the milk.



Fig;(4.38): The effect of variables of source;(Cows , Industrial's, and Human Mothers) on the Fe ion concentration in the milk.



Fig;(4.39): The effect of variables of source;(Cows , Industrial's, and Human Mothers) on the Cd ion concentration in the milk.



Fig;(4.40): The effect of variables of source;(Cows , Industrial's, and Human Mothers) on the Pb ion concentration in the milk.

4.11 - DISCOSION:

The result from test (4.1)showed that the crossing between the age of mother and her child at (M.<30Y* Ch.< 6m) in different systems of feeding , it has the maximum value of averages of calcium(421.1955 ppm) at the div System of feeding, while the minimum value of averages (221.5542 ppm) was at the most of protein system of feeding, where in iron , cadmium , and lead all so shown the maximum of each (31.7542 ppm), (2.7273 ppb), (1.41535 ppm) respectively at the div system of feeding , while the minimum value of averages of each iron and cadmium(13.9599 ppm), (0.0495 ppb) was in the most starch system of feeding, and the minimum value of averages of lead(0.2184 ppm) was at the most protein system of feeding. Generally calcium gave the maximum quantity (in ppm) while the next is the iron and the minimum one is the lead, and cadmium gave in ppb quantity more less than others. This may be for the reason that diversified food which contains vegetables, is rich in nutrient , and this may compatible with that was said by [{64}]: eating 1Kg of vegetables leads to the presence of 4mg of lead mineral in the body, and this quantity increases with eating canned food.

The result from test (4.2) showed that the crossing between the age of mother and her child at **(**M.< 30Y * Ch. 6m-1y **)** in different systems of feeding, it has the maximum value of averages of calcium (298.8108ppm) at the div system of feeding, while the minimum value of averages (82.4973ppm) was at the most starch system of feeding, where in iron ,cadmium , and lead all so shown the maximum of each(45.36315ppm),(6.73675ppb),(29.25185ppm) respectively was in the most protein system of feeding , while the minimum value of averages of each iron, cadmium , and lead(25.9585ppm),(0.2306 ppb),(0.5871ppm) respectively, was in the most starch system of feeding. This may be for the reason that they often eats a canned food which is compatible with [{64}], or it may explains that they eat rice instead of bread, compatibly with [{61}] how said; eating rice in large quantity increases the amount of cadmium in the body.

The result from test (4.3) showed that the crossing between the age of mother and her child at (M.< 30Y * Ch.>1Y) in different systems of feeding, it has the maximum value of averages of each calcium , iron , and lead(1295.1668ppm),(519.3722ppm(1.6899ppm) respectively was in the most starch. system of feeding , while the minimum value of averages of each calcium , iron , and lead(173.2209ppm),(16.1900ppm),(1.0227ppm) respectively was in the div. system of feeding , where the maximum value of averages of cadmium(1.20215ppb) was at the most protein. System of feeding, while the minimum value of averages (0.7242 ppb) was at the div system of feeding, which is may also compatible with [{61}].

The result from test (4.4) showed that the crossing between the age of mother and her child at **(**M.30-39Y*Ch.<6m**)** in different systems of feeding, it has the maximum value of averages of calcium(316.5426 ppm) at the div System of feeding, while the minimum value of averages (280.8089 ppm) was at the most protein system of feeding, and the maximum value of averages of iron (26.4104 ppm) was at the most starch System of feeding, while the minimum value of averages (5.87932 ppm) was at the most protein system of feeding, where in cadmium, and lead, also shown the maximum of each(1.7788 ppb),(2.0191ppm), respectively, was in the div. system of feeding, which is may also compatible with(Fathy,2000) , and minimum value of averages of cadmium (0.31392 ppb) was at the most protein. system of feeding, where the minimum value of averages of lead (0.73326 ppm) was at the most starch ,system of feeding, which may caused by absent of vegetable's nutrients.

The result from test (4.5) showed that the crossing between the age of mother and her child at (M.30- 39Y * Ch. 6m-1y) in different systems of feeding, it has the maximum value of averages of calcium(321.7310 ppm) at the most protein System of feeding, while the minimum value of averages (229.1980 ppm) was at the most starch system of feeding, and the maximum value of averages of iron (119.0306ppm) at the most starch . System of feeding, where the minimum value of averages (14.3819 ppm) at the most protein. Where in cadmium, and lead also shown the maximum averages of each (13.9599 ppb),(1.18967ppm) respectively at the most starch system of feeding ,while the minimum value of averages of each cadmium and lead(0.50952ppb),(0.0495 ppm) respectively, was in the div ,system of feeding, and this may caused by increases of mother's age and their childes, which is compatible with{62} research.

The result from test (4.6) showed that the crossing between the age of mother and her child at(M.30- 39Y * Ch.>1Y) in different systems of feeding, it has the maximum value of averages of each calcium , iron, and cadmium (563.1720 ppm),(232.3408 ppm),(12.1795 ppb) respectively , was in the most starch. system of feeding , while the minimum value of averages of each calcium, iron (158.3010 ppm),(13.9292 ppm) respectively , was in the div. system of feeding . where the minimum value of averages of cadmium(0.1558 ppb) at the most protein, System of feeding, and it was - maximum value of averages of lead (2.0072 ppm) at the most protein system of feeding, while the minimum value of averages (1.4558 ppm) was shown at the div system of feeding in case of lead mineral.

The result from test (4.7) showed that the crossing between the age of mother and her child at (M.≥40* Ch.< 6m) , in different systems of feeding, it has the maximum value of averages of calcium (239.3216 ppm) at the div. system of feeding, and the minimum value of averages (127.7236 ppm) was at the most protein system of feeding, while the maximum value of averages of iron(117.8965 ppm), was at the most protein system of feeding, and the minimum value of averages (16.3736 ppm) was at the div. system of feeding. Where in case of, cadmium, and lead minerals, also shown the maximum value of averages of each(0.9284 ppb),(1.07605 ppm), respectively was in the most starch. system of feeding , and the minimum value of averages of each cadmium and lead (0.0093 ppb),(0.2091ppm) respectively was in the most protein, system of feeding. The above result may also caused by increasing of age of mothers, compatibly with [62] research .

The result from test (4.8) showed that the crossing between the age of mother and her child at (M.≥40* Ch.6m-1y), in different systems of feeding, it has the maximum value of averages of each calcium, and iron(335.6212 ppm),(73.7365 ppm) respectively, at the most starch. system of feeding, while the minimum value of averages of each calcium , and iron (95.8927 ppm),(0.4802 ppm) respectively, was in the div. system of feeding, and the maximum value of averages of cadmium (0.2611ppb), was at the div. System of feeding, while the minimum value of averages (0.03443 ppb) was at the most starch system of feeding. Where the maximum value of averages of lead (0.58363 ppm), showed at the most starch system of feeding, and the minimum value of averages (0.4557 ppm) was at the div. system of feeding. And this may also compatible with the above result, showing the effect of the increasing ages of each mothers and their Childs on the concentration of cadmium and[62] lead, in the mother's body because it may be lost by repeating pregnancy and lactation.

The result in test number(4.9) showed that the crossing between the age of mother and her child at (M.≥40* Ch.>1y), in different systems of feeding, it has the maximum value of averages of calcium(196.3938ppm) at the most protein system of feeding, and the minimum value of averages (148.0189ppm) was at the most starch system of feeding, where as in iron , cadmium , and lead all so shown the maximum of each value of averages (130.4522ppm),(1.0051ppb),(1.9128ppm) respectively, was at the div. system of feeding , where the minimum value of averages of iron(7.0903ppm) was at the most starch. system of feeding, while the minimum value of averages of each cadmium and lead(0.0547ppb),(0.4672ppm) was at the most protein, system of feeding.

All above results showed the high level of concentration of Calcium and iron than the others;(cadmium, and lead) as generally it may means; the automatic needs of child for those metals during its growth. Also the accumulation of 4 metals in high level on almost systems of feeding, it may caused by reliance on canned, and readymade food without fresh; [64].

The result from test number(4.10) showed that incase of sores of milk, the maximum value of averages of calcium, and iron (883.1408ppm)(47.5766ppm) respectively, in an Industrial's, or canned milk, while the minimum value of averages of each (286.2776ppm) (42.685ppm) respectively, in mother's milk, where in case of cadmium , and lead, they shown the maximum value of averages of each (0.9216ppb),(1.0025ppm) respectively, was in mother's milk, where the minimum value of averages of each(0.0442ppb)(0.5144) was in an Industrial's, or canned milk. The high level of Ca, and Fe in canned milk, may happened for commercial's purposes, and the decreases of al 4 metals in cow's milk , it may indicates to the poor form of feeding which is depend on the physiological satiation, while the increases of each amount of cadmium, and lead in mother's milk may caused by the imbalance of Ferric during the pregnancies period; {63}.

Chapter fiver
Conclusion

5.1-Conclusion:

Generally depended on the result of this research ; the high level of concentration of Calcium and Iron than the others;(cadmium, and lead) in mother's milk as generally it may means; the automatic needs of child for those metals during its growth. Also the accumulation of 2heavy metals;(cadmium, and lead) on almost systems of feeding, it may caused by reliance on canned, and readymade food without fresh. Also the high level of Ca, and Fe in canned milk than the others sources, may happened for commercial's purposes, and the decreases of al 4 under experiment metals in cow's milk , it may indicates to the poor form of feeding which is depend on the physiological satiation, while the increases of each amount of Cadmium, and lead in mother's milk may caused by the imbalance of iron during the pregnancies period; which is compatible with{63}.

5.2-Recommendations:

Fromth I have in this thesis Irecanoded with the following:

1- mothers need to do a periodic blood tests to determine the extent of concentration of four elements in the blood in order to preserve their health and the health of their children.

2- Mothers, infants to do analyzes at the start of and during the infancy of discernible so that the necessary treatment in the case of the elements lead and cadmium in quantities toxic to the body get rid of them on the health of the baby.

3- Mothers should breastfeed because of their important benefits related to growth and be level of immunity to the child.

4- Fresh foods is batten than canned foods.

5- During pregnancy and breastfeeding should be of interest to eat, a variety of fresh vegetables which rich in mineral element sand vitamins as well as proteins and carbohydrate.

6- Farm owners and officials of the health control must concern with diet of cows, specially producing milk to away from food contaminants so as not to become a means for the transfer of toxic elements for human beings.

7- Further studies needed to identify the magnitude and severity of mineral elements in various food and dairy in particular.

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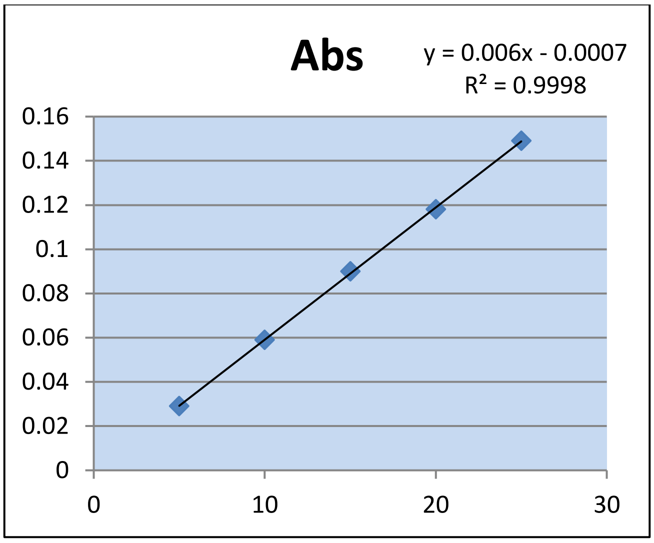
APPENDIXES:

Table (Ap6.1); The stander solution of Calcium (Ca):

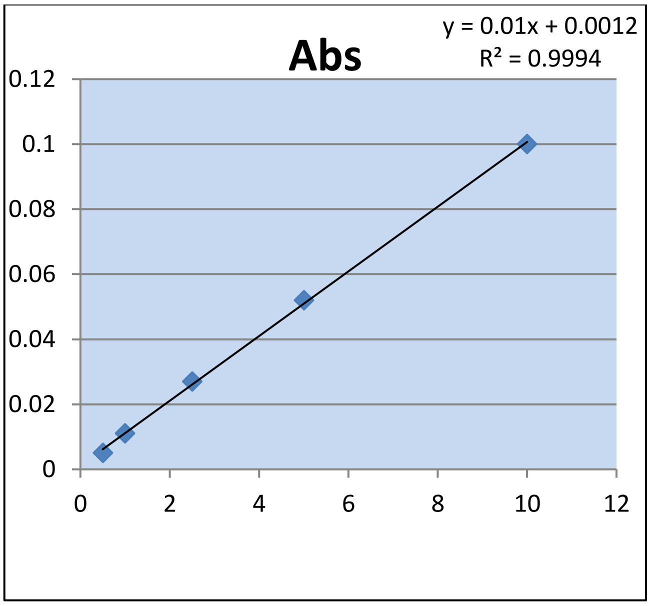
Conc	Abs	Act instr conc
5	0.029	5
10	0.059	10
15	0.0899	15.15
20	0.118	19.83
25	0.149	25



Fig;(6.1): The stander solution of Calcium (Ca)

Table (Ap6.2); The stander solution of Iron (Fe):

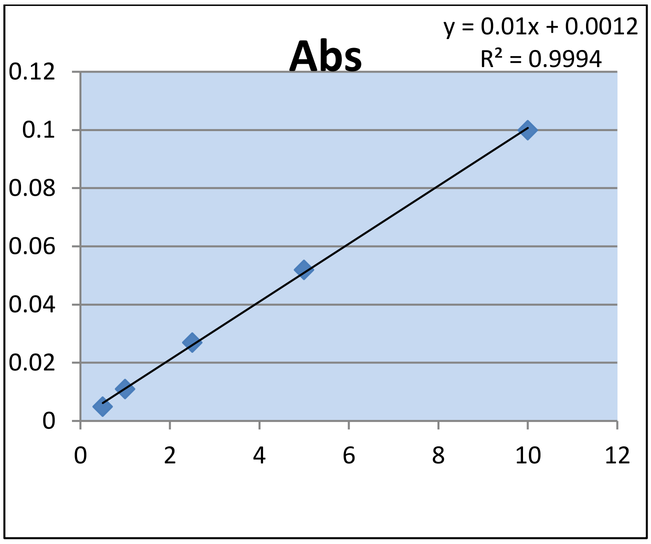
Conc	Abs	Act instr conc
0.5	0.005	0.4
1	0.011	1
2.5	0.027	2.6
5	0.052	5.1
10	0.1	9.9



Fig;(6.2):**The stander solution of Iron (Fe):**

Table (Ap6.3); The stander solution of Cadmium (Cd):

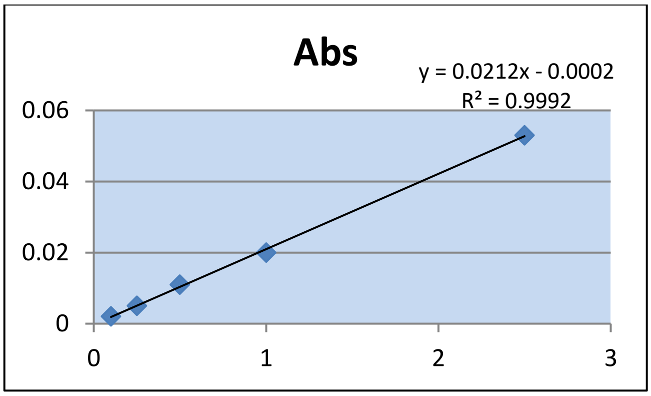
Conc	Abs	Act instr. conc
0.1	0.011	0.101
0.25	0.028	0.257
0.5	0.054	0.495
1	0.107	0.982
2.5	0.274	2.514



Fig;(6.3): The stander solution of Cadmium (Cd)

Table (Ap6.4); The stander solution of Lead (Pb):

conc	Abs	Act instr conc
0.1	0.002	0.0952
0.25	0.005	0.2381
0.5	0.011	0.5238
1	0.02	0.9524
2.5	0.053	2.5238



Fig;(6.4): The stander solution of Lead (Pb):

Table (Ap6.5); The result of collected samples analyses:

Sample No.	Weight of the sample	Ca(ppm)	Fe (ppm)	Cd (ppm)	Pb (ppb)
1	2.575	376.8359	28.1884	0.0981	0.9476
2	11.5632	172.8639	7.0493	0.005	0.2011
3	12.484	202.9944	0.3982	0.0026	0.3982
4	3.5298	301.8231	26.1608	0.0864	0.7472
5	3.1706	345.1697	26.935	1.4847	1.4303
6	3.5721	260.6674	15.4279	1.3661	0.5788
7	6.1276	196.3938	11.8803	0.0547	0.4672
8	5.3492	193.3453	12.9650	0.0519	0.3146
9	2.5581	321.5355	26.5666	1.7300	1.0056
10	8.6605	192.6884	8.5728	0.0355	0.4769
11	6.1161	205.6073	12.920	0.9406	0.7983
12	3.5317	394.5281	20.9177	0.7857	1.0816
13	2.0053	432.058	30.7747	0.1558	2.0072
14	1.0568	757.7924	58.6014	0.3832	3.2094
15	5.5387	276.414	12.953	0.4658	0.7551
16	7.7195	221.7459	11.0318	0.0052	0.0891
17	4.9446	209.8719	10.6338	0.0051	0.4015
18	1.8793	870.2575	35.3975	0.0146	30.1543
19	4.2253	284.7526	17.3751	0.0118	0.1734
20	2.5904	395.0384	29.8632	2.3191	2.1464
21	4.5385	245.0997	14.3610	0.6715	0.7337
22	4.6638	233.8758	16.5139	0.6733	0.4974
23	7.4886	256.0489	9.6807	0.8072	0.9271
24	7.1411	242.4746	10.1325	0.0039	0.0364
25	7.0984	253.4994	12.2774	0.0074	0.1324
26	2.7051	241.1879	39.2476	0.1238	0.9454
27	4.0169	4.0169	92.0131	0.0542	0.4967
28	1.1542	1.1542	440.3721	2.2137	0.3903
29	1.4648	1.4648	43.6237	2.7973	2.9048
30	4.0856	4.0856	16.0129	0.6480	0.6737
31	4.4835	4.4835	22.3018	1.1838	1.220
32	2.8501	2.8501	55.6174	1.9385	2.0499
33	1.9097	1.9097	48.9205	0.0498	0.5996

Sample No.	Weight of the sample	Ca(ppm)	Fe (ppm)	Cd (ppm)	Pb (ppb)
34	0.5033	1152.4786	132.6247	0.8792	2.2601
35	3.4806	270.1941	17.7017	0.0181	0.1942
36	1.9134	1339.601	31.7864	0.0322	0.2285
37	2.8051	258.0666	27.8413	3.1389	18.9236
38	2.4825	431.5861	18.1511	0.1108	0.9849
39	1.1252	157.8697	28.4505	2.6906	3.9326
40	0.2850	1164.4211	268.6754	0.1667	5.6491
41	0.9511	400.1051	75.7833	0.0762	1.5745
42	1.3529	2288.033	1009.3484	0.0407	0.0718
43	1.7719	296.0861	31.2673	0.1721	1.4786
44	1.3000	140.5692	48.9000	1.2558	3.0885
45	4.2857	261.6533	15.0413	0.7076	1.0378
46	0.7066	254.1254	74.7134	12.8255	57.830
47	4.2431	213.7712	15.6872	0.6729	0.9321
48	2.1814	307.3359	38.614	0.0195	0.3748
49	6.2016	168.0881	10.5424	0.0331	0.2991
50	1.4463	465.5552	46.9923	3.6870	22.2119
51	14.5144	136.6651	5.0269	0.0227	0.1014
52	7.0510	185.0149	7.7542	0.0046	0.0465
53	1.1570	556.9144	59.3107	0.0821	1.4434
54	2.7297	302.3006	29.3961	2.2594	3.3081
55	0.4749	1187.129	123.9366	12.7974	10.9549
56	1.3885	414.775	165.1945	0.0720	1.2027
57	1.5125	430.6496	100.5306	0.3669	1.8331
58	0.5398	542.5112	134.2025	6.6228	3.8023
59	5.5868	157.081	13.4437	0.4475	0.7818
60	1.1882	304.5594	74.6318	9.6006	33.9589
61	0.8570	153.489	73.4393	0.0671	1.4732
62	1.8878	283.3735	44.6115	0.0278	0.3867
63	9.4646	324.3933	8.8062	0.01796	0.2229
64	0.2418	563.1720	232.3408	12.1795	1.5767
65	5.3951	152.885	11.3015	0.0102	0.1887
66	1.4368	168.3150	187.4652	2.262	3.3181
67	2.0090	146.4485	3.0289	0.1916	1.6762
68	2.6506	43.4448	22.0931	1.1922	1.7506
69	3.1186	88.1309	20.1020	1.7676	1.8831

Sample No.	Weight of the sample	Ca(ppm)	Fe (ppm)	Cd (ppm)	Pb (ppb)
70	5.1901	89.1462	11.0268	0.6864	0.9471
71	2.1796	90.4306	1.2606	0.1629	1.5496
72	2.7058	40.5416	0.3326	0.0776	0.7512
73	1.3786	49.8694	32.6454	4.0149	5.8973
74	5.8865	127.7236	117.8965	0.0093	0.2091
75	1.5846	109.3935	9.7564	0.0615	1.0713
76	5.1203	39.0724	12.1507	0.0771	0.5621
77	8.7311	91.0097	1.2078	0.0447	0.3353
78	7.0647	78.0507	4.9503	0.0149	0.2511
79	3.8769	173.2209	16.1900	0.7242	1.0227
80	10.7356	95.8927	0.4802	0.2611	0.4557
81	5.6550	158.301	13.9292	0.9757	1.4458
82	3.6391	73.1506	0.5365	0.0223	0.2672
83	1.9766	88.8002	12.2420	0.0379	0.7627
Mb1	5	371.0765	52.0530	0.078	0.5905
Mb2	5	387.7645	0.6195	0.605	0.667
Mb3	5	647.993	1.4820	0.557	0.8515
Mb4	5	607.0175	0.6860	0.009	0.1795
M1	5	953.4195	42.0165	0.0185	0.3395
M2	5	846.5625	28.7165	0.0525	0.5495
M3	5	980.492	60.6165	0.0505	0.5025
M4	5	753.1605	40.0940	0.0551	0.6665

*MB means Cow's milk samples , M means canned milk samples