Amount of Zinc Transferred in Breast Milk to Breastfed Moroccan Babies with Normal or Low Birth Weight at 1, 3 and 6 Months After Birth

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Abstract: The amount of zinc in breast milk is generally regarded as sufficient to cover the increasing zinc demands of most infants. However, this is not well investigated where stores zinc may be compromised in babies with low birth weight (LBW) who are born with low stores of zinc. In Morocco, this is the first time that the amount of zinc transferred in breast milk has been estimated. This study included 32 mother-baby pairs. In our case study, we aimed to measure The quantity of zinc in mothers' breast milk with normal birth weight (NBW) and LBW babies who were exclusively or not exclusively breast fed at 1,3 and 6 month after birth. The results showed that the majority of mothers have a BMI ≥25 kg/m^2 this means that all mothers are overweight during 6 months after birth. Zinc concentration (mg/l) in mothers' breast milk decreased from first month to six month. p- value showed that for mothers with NBW babies, there is a significant difference between the 1 and 6 month (p=0.0003) and between 3 and 6 month after birth (p=0.0007). For mothers with LBW babies, p-value showed a significant difference between the zinc concentration in breast milk in the 1st and 3rd month (p=0.0007), 1 and 6 month (p< 0.0001) and between 3rd and 6th month after birth (p=0.0056). The rate of NBW babies who were exclusively breastfed was 36.67%, 30.25% and 10% successively in 1st, 3rd and 6th month after birth. For LBW babies, the rate of exclusively breastfed was 15.38%, 7.69% and 2.69% successively in 1st, 3rd and 6th month after birth. Based on the K. Brown study in 2009, we can develop a mathematical equation to our own population using our data: Ln [Zinc] = 0.960 – 0.161*Ln(âge) – 0.187*Ln(âge)^2. In conclusion the zinc concentration in milk is within normal range and decreases with the age of the babies. The predicted model of zinc concentration in breast milk was developed and tested.

Keywords: Zinc in breastmilk, LBW, Exclusive breastfeeding, Model of zinc concentration.

INTRODUCTION

Adequate zinc nutrition is essential for human health because of zinc's critical structural and functional roles in multiple enzyme systems that are involved in gene expression, cell division, growth, immunologic and reproductive functions [1]. One-third of the world's population lives in very exposed countries with the zinc deficiency [2]. The most vulnerable groups are babies, young children, pregnant women and nursing. This is due to their high need for this essential nutriment [3] The recent Lancet series on maternal and child undernutrition concluded that zinc deficiency is responsible for ~4% of child mortality and disability-adjusted life-years [4]. In addition, Low birth weight is associated with growth faltering and substantially increasing risk of severe infection–related morbidity and mortality. Impaired zinc status has been reported in small-for-date infants [5]. Breast milk is the only dietary source of zinc for breastfed young infants, and it remains a potentially important source of zinc for older infants and young children who continue breastfeeding beyond early infancy [6]. The colostrums is very rich in zinc, it is gradually replaced by mature milk, towards the end of the first month of life, the content zinc of the mother's milk becomes very weak and more lactation advances, plus the zinc rates are low in milk. However the needs for growth are accompanied by a significant consumption by zinc during the first year by life [7]. There is very little information on zinc intake from breast milk in infant from developing countries. In Morocco, there is no data on the prevalence of the zinc deficiencies. Furthermore, significant reduction in zinc concentrations during lactation is reported, and it has been postulated that the amount of zinc provided by breast milk may be lower than the Recommended Dietary Allowance (RDA) of zinc [8]. The objective of our study is to estimate the amount of zinc transferred in breast milk to breastfed babies with normal or low birth weight at 1, 3 and 6 months.

MATERIALS AND METHODS

Subjects and Study Design

This investigation is a longitudinal study conducted on 32 mother/baby pairs. Twelve of them were with

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E-ISSN: 1929-4247/14

LBW babies and twenty with NBW ones. Women (between 18 and 49 years old) were recruited before the childbirth, during the third antenatal consultation (38 week of amenorrhea), by a pediatric, in the maternity of the children Hospital in Rabat, Morocco. They had similar socioeconomic level without a history of serious disease. They delivered a healthy full-term infant. To include these mothers in this study, they must breastfeed their babies until the sixth month. Mother-infant pairs visited the maternity one month after delivery. We used a questionnaire to collect the data about the mothers and the babies. Mothers' and infants' weight and height were measured. Mothers' breast milk was collected. All these operations were repeated in 3^{rd} and 6^{th} month after childbirth.

Determination the Concentration of Zinc in Mother's Milk

Collect, Transport and Storage of the Samples

Collection of the mother's milk samples (2-4 ml) for each participating woman, after washing hands well. Begin nursing infant. Approximately mid-feed (2-3 minutes into the feed), remove infant from breast to collect a sample, by using a car milk or all simply by pressing the breast, to facilitate the rise of milk at 1st, 3^{rd} and the 6^{th} month after the birth. The samples are placed in a kept refrigerator with approximately -4°C and transported to the laboratory. Then aliquots' and stored in the freezer at -20°C until the moment of the analysis.

Preparation of the Sample for the Analysis

Preparation of the Milk Samples

After acid washed all the materials to ensure that they are zinc-free. We start by Defrost milk sample and keep warm in water bath. Weigh empty beaker without watch glass. Record weight and label beaker. Vigorously shake milk sample and immediately transfer entire sample into weighed beaker. Weigh beaker and milk sample. Record weight. Cover beaker with watch glass. With each set of samples, prepare internal control standard as above using milk pool. Also include a blank using an empty beaker. Place beaker into drying oven at ~100 º C until completely dry. Once dry, transfer beaker with milk sample into muffle furnace and heat at 200 º C for 3 hours, 300 º C for 3 hours and finally for 24-hours at 450 º C. Let samples cool and remove from furnace. Add enough concentrated nitric acid to just cover ashes. Place on hot plate at a very low temperature (not boiling) until sample is dry again. Return sample to muffle furnace for 24 hours at 450^{o}C.

Reconstitution

Quantitatively reconstitute washed sample with 0.125 N HCl by first rinsing the underside of the watch glass and allowing the rinse to fall into the beaker. Aspirate reconstituted sample with a transfer pipette and transfer into a 10 ml volumetric flask. Rinse sides of beaker with another small volume of 0.125 N HCl. Using transfer pipette, continue to thoroughly rinse the sides of the beaker with a small new volume of HCl. Aspirate and release the HCl in the beaker a few times, then transfer into volumetric flask. Repeat steps until a volume of exactly 10 ml is reached in the volumetric flask (sample is complete when bottom of liquid meniscus lies atop the white line drawn around the circumference of the flask's neck). Label 14-ml polypropylene test tube (Sarstedt) with subject information (Subject ID, date and time of sample, reconstitution volume). Cap volumetric flask, shake well and immediately pour into labeled test tube. Samples are ready for zinc determination by AAS analyses using standard procedures.

Milk Zn concentration is calculated with the following equation:

[Milk Zn] = μg Zn/dl (AAS) * dl/100 ml * dilution factor * total reconstitution volume (ml)/aliquot weight (g)

Analyzes

The zinc content in mother's milk is determined by the technique of analyses per atomic emission: Inductively coupled plasma mass spectrometry «ICP-MS», after Quality control process for zinc analysis by in expert for University of Chalmers (Sweden).

Statistical Analyze

Data were analysed using Epi-info version 6 and MedCalc 11.5.1. software. Independent two sample t-tests were used assess the significance of the difference between means. Significance ($p < 0.05$) was determined at the ninety five per cent confidence level.

RESULT

Mothers Anthropometric Characteristics According to Babies' Age

Table **1** showed that BMI (Body Mass Index) for mothers with NBW babies (n=20) passes from 26.8±3.6kg/m^2 in first month to 25.8±4.0kg/m^2 in third month and finally reaches 25.8±2.6kg/m^2 at 6 month.

Table 1: Maternal Anthropometric Characteristics at One, Three, and Six Months Postpartum (n=32)

	Babies age	Age (year)	Height (cm)	Weight (Kg)	BMI (kg/m^2)
Mothers with NBW babies (n=20)	1month	28.0±5.9	158.0±0.05	66.2±10.9	26.8±3.6
	3month			63.7±11.2	25.8±4.0
	6month			63.5±7.9	25.8±2.6
Mothers with LBW babies (n=12)	1month	25.6±6.6	157.0±0.05	65.3±11.2	26.2±4.5
	3month			62.2±10.7	24.9±4.2
	6month			63.0±9.6	25.3±3.9

For mothers with LBW babies (n=12) the BMI passes 26.2±4.5 kg/m^2 in first month to 24.9±4.2 kg/m^2 in third month and finally gets to 25.3±3.9kg/m^2 at 6 month. The results showed that the majority of mothers have a BMI ≥25 kg/m^2 that means that mothers with LBW or NBW babies are at the beginning limit of overweight during 6 months after birth.

Babies' Anthropometric Characteristics by Age (n=32)

Babies' anthropometric data showed that they did not suffer from any form of malnutrition (Z-scores between -1.43 and 1.13) and they grew normally during the six months of the study (Table **2**).

Zinc Concentration (mg/l) in Breast Milk for Mothers with NBW and LBW Babies During 6 Month After Birth (n=32)

Zinc concentration (mg/l) in breast milk for mothers decreased from first month to six month as would be expected in Table **3**. For mothers with NBW babies, the zinc concentration in breast milk was 2.674±1.239mg/l, 2.037±0.714mg/l and 1.109±0.474mg/l successively in 1st, 3rd and 6th month after birth. p-value showed a significant difference between the zinc concentration in breast milk between the 1 and 6 month (p=0.0003) and between 3 and 6 month after birth (p=0.0007). As far as mothers with LBW babies, the zinc concentration in breast milk was 2.524±0.992mg/l, 1.754±0.673mg/l and 1.233±0.752mg/l successively in 1st, 3rd and 6th month after birth. p-value showed a significant difference between the zinc concentration in breast milk in the 1 and 3 month (p=0.0007), 1 and 6 month (p< 0.0001) and between 3 and 6 month after birth (p=0.0056). However there was no significant difference in breast milk concentration between mothers with NBW babies and mothers with LBW babies in 1st, 3rd and 6th month after birth.

The Rate of Zinc (mg / l) in Breast Milk by Mode of Lactation

After quantifying breast milk and other fluids consumed by infants during the first six months after birth using the isotope stable method [9], we found that the rate of NBW babies who were exclusively breastfed was 36.67%, 30.25% and 10% successively in 1st, 3rd and 6th month after birth. For LBW babies, the rate of exclusively breastfed babies was 15.38%, 7.69% and 2.69% successively in 1st, 3rd and 6th month after birth.

Zinc Concentration (mg/l) in Breast Milk for Exclusively Breast Feed Mothers with NBW and LBW Babies During 6 Month After Birth (n=32)

Using the deuterium oxide dose-to-mother technique [9], zinc concentration (mg/l) in breast milk for mothers with NBW babies how exclusively breast

Table 2: Babies' Anthropometric Characteristics by Age (n=32)

	Babies with NBW (n=20)			Babies with LBW (n=12)		
Babies age (month)	1	3	6	1	3	6
Height (cm)	51.8±2.5	60.0±2.1	68.0±5.4	50.5±2.7	59.1±2.9	67.1±7.9
Weight (kg)	4.00±0.38	5.95±0.65	7.86±0.78	3.60±0.70	5.74±1.08	7.34±1.45
Z-score weight/Lenght	0.25±1.20	-0.19±1.40	-0.05±1.18	-0.02±0.57	-0.16±0.95	-0.53±1.20
Z-score weight/Age	-0.73±1.31	-0.28±0.89	0.51±1.28	-1.43±1.50	-0.64±1.21	-0.02±0.32
Z-score lenght/Age	-1.01±1.27	-0.15±0.78	1.13±1.87	-1.61±1.23	-0.68±1.41	0.78±0.84
Z-score BMI/Age	-0,29±0.72	-0.26±1.07	-0.17±1.10	-0.82±1.38	-0.35±0.65	-0.64±1.84

Table 3: Zinc Concentration (mg/l) in Breast Milk for Mothers with NBW and LBW Babies During 6 Month After Birth (n=32)

Zinc in breast milk (mg/l)			
Babies age	**Mothers with NBW babies (n=20)**	**Mothers with LBW babies (n=12)**	**p-value**
1 month	2.674±1.239	2.524±0.992	0.6731
3 month	2.037±0.714	1.754±0.673	0.2181
6 month	1.109±0.474	1.233±0.752	0.5863
p-value	1-3: 0.1213 1-6: 0.0003 3-6: 0.0007	1-3: 0.0007 1-6: < 0.0001 3-6: 0.0056	

P(<0.05).

fed was (Table **4**) 2.764±2.441mg/l, 1.168±0.000mg/l and 0.705±0.173mg/l successively in 1st, 3rd and 6th month after birth, p-value showed a significant difference between the zinc concentration in breast milk between the 1st and 3rd month (p=0.0392) and between 1 and 6 month after birth (p=0.0057). Regarding the exclusively breast fed mothers with LBW babies, the zinc concentration in breast milk was 2.457±1.408mg/l, 1.662±0.552mg/l and 0.955± 0.598mg/l successively in 1st, 3rd and 6th month after birth, p-value showed a significant difference between the zinc concentration in breast milk in the 1st and 3rd month (p=0.0001), 1st and 6th month (p< 0.0001) and between 3rd and 6th month after birth (p=0.0063). However there was no significant difference in breast milk concentration between mothers with NBW babies and mothers with LBW babies in 1st, 3rd and 6th month after birth.

Kinetic of Zinc Concentration in Breast Milk According to Babies' Age

Based on the Kenneth Brown study in 2009 [10]: the amount of zinc (mg / l) in breast milk and baby`s age, we can develop a mathematical equation to our own population (Figure **2**). the data were normalized by logarithmic transformation: Ln [Zinc] = 0.960 – 0.161*Ln(âge) – 0.187*Ln(âge)^2. This shows that if we have babies age, we can calculate the amount of available zinc in breast milk.

Test the Reliability of our Equation with Brown's Models

In order to use the distribution of zinc concentration in breast milk according to babies age model for our population, we conducted a comparison of results obtained by the two models.

Table **5** shows that there is not a significant difference between the two equations. Our equation is acceptable and usable for our population.

Model 1: Morocco.

Ln [Zinc] = 0.960 – 0.161*Ln(age) – 0.187*Ln(age)2

Model 2: K. Brown et *al*. (2009) [10]. Focus on a synthesis of 33 international studies.

Ln [Zinc] = 0.975 – 0.501*Ln(age) – 0.063*Ln(age)2

Table 4: Zinc Concentration (mg/l) in Breast Milk for Exclusively Breast Feed Mothers with NBW and LBW Babies During 6 Month After Birth (n=32)

	Zinc in breast milk (mg/l)			
	Mothers with NBW babies (n=20)		**Mothers with LBW babies (n=12)**	
	Exclusively breast feed	**Non exclusively breast feed**	**Exclusively breast feed**	**Non exclusively breast feed**
1 month	2.764±2.441	2.657±1.116	2.457±1.408	2.560±0.712
3 month	1.168±0.000	2.110±0.694	1.662±0.552	1.805±0.739
6 month	0.705±0.173	1.183±0.477	0.955±0.598	1.263±0.769
p-value	1-3: P = 0.0392 1-6: P = 0.0057 3-6: P = 0.1130	1-3: P = 0.1465 1-6: P = 0.0002 3-6: P = 0.0006	1-3: P = 0.0048 1-6: P < 0.0001 3-6: P < 0.0001	1-3: P = 0.0001 1-6: P < 0.0001 3-6: P = 0.0063

P(<0.05).

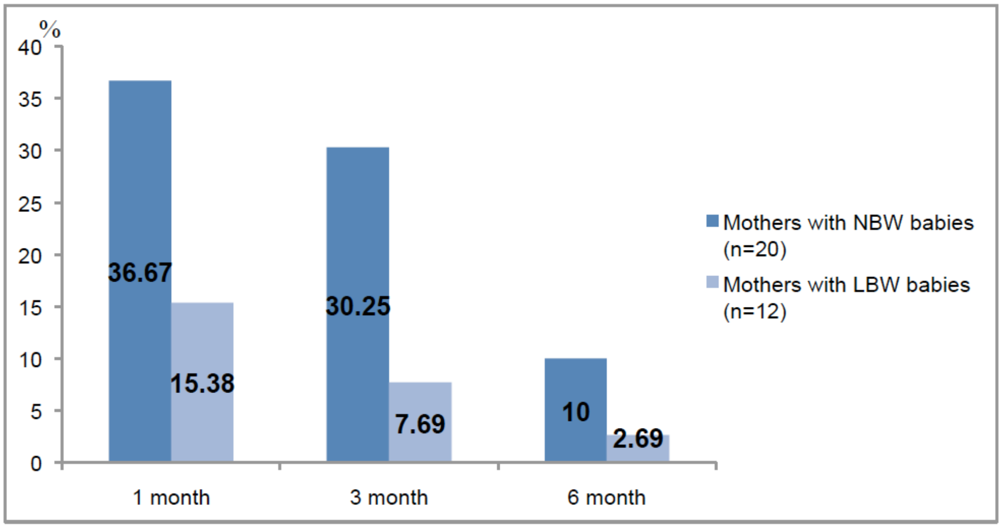

Figure 1: The rate of zinc (mg / l) in milk by mode of lactation.

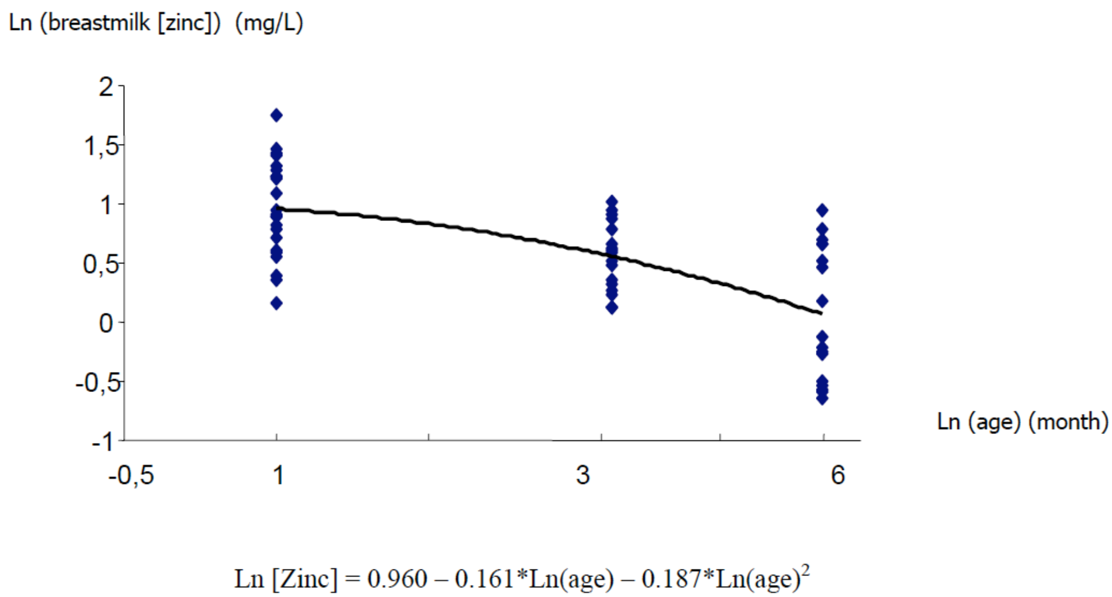

Figure 2: Distribution of zinc concentration in breast milk according to babies' age after mathematical transformations of data.

Table 5: Comparison of Experimental Data and those Obtained After the Application of the Two Models

	Experimental Data (mg/l) (n = 32)	Equation 1 (Morocco) (mg/l)	Equation 2 (Brown and al. 2009) (10) (mg/l)	(1) – (2) (mg/l)
1 month	2.61	2.61	2.65	0.04
3 month	1.74	1.45	1.33	0.12
6 month	1.07	1	0.86	0.14

DISCUSSION

The amount of zinc in breast milk is generally regarded as sufficient to cover the increasing zinc demands infants [11]. However, this is not well investigated where stores zinc may be compromised in babies with low birth weight (LBW) who are born with low stores of zinc [12]. Breast milk zinc concentration declines rapidly during the first few months postpartum and more slowly thereafter. It is the important source of highly bioavailable zinc during this period of life and may be adequate as the sole source of zinc for exclusively breastfed term infants until ~6 months [10]. In Our study the results showed that zinc level decreased with increasing babies' age. Zinc concentrations in the breast milk of mothers who delivered LBW babies are slightly low compared with mothers who delivered NBW. We found that zinc breast milk concentration for mothers with NBW babies was 2.674±1.239mg/l, 2.037±0.714mg/l and 1.109±0.474 mg/l successively in 1st, 3rd and 6th month after birth. Regarding the mothers with LBW babies the zinc concentration in breast milk was 2.524±0.992mg/l, 1.754±0.673mg/l and 1.233±0.752mg/l successively in 1st, 3rd and 6th month after birth. However there was no significant difference in breast milk concentration between mothers with NBW babies and mothers with LBW babies in 1st, 3rd and 6th month after birth. Our results show that during the sixth months after birth, there was not an impact of the zinc amount in breast milk on the babies' weight. Therefore the LBW babies recovered their weight quickly and they did not suffer from any form of malnutrition. Sellen DW and Piletz JE [12-13] found similar results. Breastfeeding is the best method of nutrition for infants. The World Health Organisation (WHO) recommends breastfeeding as the exclusive method of nutrition for newborns and infants at least up to 6 months of age [14]. In Morocco, there has been an alarming decline in the practice of exclusive breastfeeding (EB) over the past fifteen years. Based on questionnaire analysis, the proportion of exclusive breastfeeding until 6 months is currently around 15% [15]. Our study revealed that the rate of NBW babies who were exclusively breastfed was 36.67%, 30.25% and 10% successively in 1st, 3rd and 6th month after birth. For LBW babies the rate of exclusively breastfed babies was 15.38%, 7.69% and 2.69% successively in 1st, 3rd and 6th month after birth. In other countries such as Cambodia (60.1%), Germany (45%), Indonesia (39%), Syria (38%) and Switzerland (32%) [16-17], exclusive breastfeeding is practiced by a large proportion of women. Zinc concentration (mg/l) in breast milk for mothers with NBW babies who are exclusively breastfed was 2.764±2.441mg/l, 1.168±0.000mg/l and 0.705±0.173 mg/l successively in 1st, 3rd and 6th month after birth. For mothers with LBW babies, the zinc concentration in breast milk was 2.457±1.408mg/l, 1.662±0.552mg/l and 0.955±0.598mg/l successively in 1st, 3rd and 6th month after birth. However there was no significant difference in breast milk concentration between mothers with NBW babies and mothers with LBW babies in 1st, 3rd and 6th month after birth. This is confirmed by the study of K. Brown [18] P. Hemalatha [19] A. Higashi [20] PB. Moser [21]. The amount of zinc transferred in breast milk to partially breastfed infants less than 6 months of age is approximately 15% less than that described above for exclusively breastfed infants, because of the smaller volumes of milk consumed by partially breastfed infants [10]. The age-related pattern of change in milk zinc intakes is similar for both groups of infants. Ni environmental or constitutional variables consistently affected zinc concentration or its rate of decrease in breast milk. Stage of lactation is the only variable associated with important changes in milk zinc concentrations [22]. On the other hand, several longitudinal studies have found greater variability in milk zinc concentration between women than within women [23], indicating that individual women have characteristic levels of zinc in their milk, possibly because of genetic factors influencing zinc transport to or within the mammary gland. Based on the Kenneth Brown study in 2009 [10]: the amount of zinc (mg / l) in breast milk and baby`s age, we can developed a mathematical equation to our own population. The data were normalized by logarithmic transformation: $\text{Ln}[\text{Zinc}] = 0.960 - 0.161*\text{Ln}(\text{âge}) - 0.187*\text{Ln}(\text{âge})^2$. This shows that if we have babies age, we can calculate the amount of available zinc in breast milk. Our equation was tested and used.

CONCLUSION

Zinc concentration in breast milk for both groups of mothers is within normal range and decreases with the age of the babies. Therefore the LBW babies recovered their weight quickly and they did not suffer from any form of malnutrition. The rate of exclusively breast feeding babies is very low; there has been an alarming decline in the practice of exclusive breastfeeding in Morocco. The predicted model of zinc concentration in human milk was developed and tested.

ACKNOWLEDGEMENTS

The financial and technical support of the International Atomic Energy Agency is gratefully acknowledged. We are grateful to the entire staff for their kind collaboration.

We are also indebted to the mothers of the infant who agreed to participate in this study.

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Received on 01-02-2014 Accepted on 25-02-2014 Published on 10-03-2014

http://dx.doi.org/10.6000/1929-4247.2014.03.01.6