Journal of Toxicology and Environmental Health Sciences Vol. 3(11), pp. 317-327, 5 October, 2011
Available online at http://www.academicjournals.org/JTEHS
ISSN 2006-9820 ©2011 Academic Journals

Full Length Research Paper

Assessment of sorghum beer for alcohol and metal ions content and genotoxicity in mice bone marrow

Asita A. O.[1]*, Tanor E. B.[2], Magama S.[1] and Khoabane N. M.[2]

[1]Department of Biology, National University of Lesotho, P. O. Roma 180 Maseru, Lesotho, Southern Africa.
[2]Department of Chemistry, National University of Lesotho, P. O. Roma 180 Maseru, Southern Africa.

Accepted 3 October, 2011

National well-being, life expectancy (LE), water quality, alcohol consumption etc, have been shown to be interrelated. In Lesotho, with LE below world average, one widely consumed beverage is a locally brewed sorghum beer, *sesotho*. Therefore, beers from four different sources were analyzed for metals, alcohols (mg/L) and mice bone marrow genotoxicity. The beers contained different ($p<0.05$) concentrations of As (0.012 to 0.059), Co (0.127 to 0.160), Cr (0.052 to 0.069), Cu (0.004 to 0.057), Fe (0.070 to 0.600), Ni (0.004 to 0.031), Pb (0.019 to 0.029), Se (0.645 to 0.942) and Zn (0.317 to 6.337). Cd (0.032 to 0.035), and Hg (0.131 to 0.150) concentrations were not different ($p>0.05$). The concentrations, in the beers, of Co, Cr, Cu, Fe, Ni and Pb were lower ($p<0.05$), while those of Se, As, Cd, Hg, Se As, Cd, Se and Zn were higher ($p<0.05$), than in the water used for brewing. Alcohol concentrations (mg/L) in the beers differed significantly ($p<0.05$) and were, total alcohol (53,000 to 74,200), methanol (800 to 2000) and ethanol (53,000 to 74,000). Beer from one source only was assessed for genotoxicity. It was nonclastogenic but toxic at 50% concentration only.

Key words: Sorghum beer, metals, alcohol, genotoxicity.

INTRODUCTION

The use of the gross domestic product (GDP) as a standard gauge of national economic health is being reexamined, mostly by development economists, most of whom now favour alternative yardsticks that combine indicators of human well-being, such as health, population, and wealth, with those of environmental sustainability (water quality, species diversity, and energy use) to generate a more integrated picture of the general wellbeing of nations and the world (Bourgeault-Tassé, 2011). Thus, interrelationships among some of the key indicators of well-being, such as life expectancy, human and environmental health and economic growth have been suggested (Aísa and Pueyo, 2004). Life expectancy can fall due to problems like famine, war, disease and poor health. The higher the life expectancy, the better socio economic conditions a country is in. For instance, the 2009 figures for life expectancy at birth for males and females in Lesotho were put at 46 and 50 years, respectively whereas similar figures for males and females for the following developed countries were given as; USA (76 and 81), Germany (78 and 83), Norway (79 and 83) and Japan (80 and 86), respectively (WHO, 2011). Life expectancy was shown to correlate negatively with the behavioral factor of alcohol consumption, with larger amounts of alcohol consumption being related to slightly lower life expectancy (Organisation for Economic Co-operation and Development – OECD, 2006). Using information for 161 different countries, the lower life expectancy of males correlated with the higher per capita, alcohol consumption and cirrhosis of the liver deaths among males (Templer et al., 1993).

Water quality, as one aspect of the wider theme of environmental sustainability, is also related to the general well-being of individuals and nations and metal ions in water and beverages are of concern to many regulatory authorities. The widespread roles of metal ions in health and disease range from the requirement for intake of essential trace elements to toxicity associated with metal overload. Many metal ions are associated with enhanced oxidative stress, inflammation and cancer (Valko et al., 2006; Halliwell and Gutteridge, 2007).

Despite regulatory controls, numerous sources of metal

*Corresponding author. E-mail: ao.asita@nul.ls, aoasita@yahoo.co.uk. Tel: +266 22213292, +26663060354. Fax: +266 22 340000.

ingestion have been recently reported including contaminated drinking water, seafood, breast milk, herbal medicines, smoking, together with plants and animals used in the diet (Wang et al., 2005; Ang et al., 2005; Sharma and Agrawal, 2005; Navarro and Alvarez, 2005; Zheng et al., 2007).

In Lesotho, indigenous fermented beers from sorghum (*Sorghum bicolor*) are the traditional drinks and are widely consumed. A survey in 1983/1984 found that between 40 to 60% of rural households in some districts in Lesotho brew and/or sell sorghum beer, which has traditionally lubricated much of the social and economic fabric of *Basotho* life such as facilitating *matsema* or work parties or by providing refreshments for rites and feasts connected with the ancestors (Senaoana et al., 1984). The occupation of beer brewing was connected with women migrants who brewed and sold beer illegally, thereby commercializing the traditional beer (Bonner, 1990). As migration rates increased, Basotho women settled in towns in Lesotho to brew and sell beer to migrants traveling to and from South Africa and local labourers (Larsson et al., 1998). The beer, which is called *sesotho* in Lesotho is prepared by boiling a mixture of germinated sorghum grains, warm water and maize meal in a metal drum, followed by cooling in the open air to produce a concoction called *sesotho*. To this concoction is added the inoculums of either yeast or a mixture of previously used sorghum which contains some wild yeast which ferments the sugars. The whole slurry mixture is kept in a warm place so that efficient fermentation is achieved (Larsson et al., 1998).

A survey that was carried out in 2000 of households in 29 villages in Lesotho showed that 26% of adults consumed alcohol and of the consumers, 65% reported consuming traditional home-brewed beer exclusively, with another 20% drinking both western-type and traditional beer (Siegfried, 2001). The daily volumes of traditional beer that were consumed on a typical drinking day ranged from 340 to 10 000 ml with a mean (SD) of (2634 ± 1737 ml) (Siegfried, 2001). In 2004, alcohol use disorders (15 + years) contributed to 1.35% of health problems among men and 0.15% among women in Lesotho (WHO, 2011).

The aim of this study therefore, was to assess the locally brewed sorghum beer and the water used for brewing from four different sources for the content of metal ions, alcohol, and *in vivo* mouse bone marrow erythropoietic cell toxicity and genotoxicity.

MATERIALS AND METHODS

Beer sample

The beer and water samples were collected from four brewing locations within the Maseru District of Lesotho, namely, Mafefooane, Mahlanyeng, Sea Point and Mafikeng. Sea Point is asuburb in Maseru city, while the other three locations are settlements in the periphery of the city within a 40 km radius.

Preparation of the beer samples

The beer samples were individually filtered through 8 layers of cheese cloth to remove large particulate matter, stored in a freezer and used throughout the duration of the experiment. Each filtered beer sample was further filtered using a vacuum pump, Rocker 400 by INSTRUVAC® of South Africa.

For the determination of metal ions, the temperature of the filtered beer sample was adjusted to 20 to 25°C and decarbonated by transferring the sample into a 250 ml Erlenmeyer flask with shaking, first gently and then vigorously, until all gas had been released. The sample was then filtered through Whatman No. 1 filter paper. A volume of 100 ml of the filtrate was transferred into a 250 ml beaker, 1.0 ml concentrated HNO_3 and 0.5 ml concentrated HCl were added and heated gently on a hot plate without boiling until the volume was reduced to 20 ml. The solution was refluxed for 30 min and cooled, transferred into a 50 ml graduated flask and diluted to the mark with deionized water.

Quantification of heavy metals

Preparation of solutions of heavy metals

All the reagents and chemicals used were high analytical grade and supplied by Merck, Johannesburg, South Africa. The stock solutions for the metals analysis were prepared according to the Varian AAS Handbook, 1998.The stock solutions contained 1000 mg/L of metal ion.

Determination of metal ions concentration

The concentrations of As, Cd, Co, Cr, Cu, Fe, Hg, Ni and Se in the beer and water samples were determined by induced coupled plasma atomic emission spectrophotometer (ICP-AES Varian Liberty AX).

The concentrations of Pb and Zn were determined by flame atomic absorption spectrophotometer (AAS, Varian spectra AA 220FS) as it gives netter detection for those metals (VFAASAM, 1989). Five standard solutions of each of the metal ions were prepared and calibration graphs constructed for determining the concentration of the metal ions in the samples.

Determination of percentage of total alcohol concentration (in v/v)

The total alcohol concentration was determined according to the back titration method 28.1.07 of the association of official analytical chemists, (AOAC, 1995).

The distillate of the beer sample was titrated with a standard ferrous ammonium sulphate. Blank titration was done by titrating 25 ml of the dichromate solution with ferrous ammonium solutions. The percentage of total alcohol concentration (in v/v) was calculated using the following equation:

$$\%\ Alcohol = 25.00 - \left(25.00\ x\ \frac{V_1}{V_2}\right)$$

Where, 25.00 was the amount of the dichromate solution used to collect the distillate, V_1 was the amount of the Fe^{2+} used to titrate the distillate and V_2 the amount of Fe^{2+} used in the blank titration.

Analysis of beer for different alcohols by nuclear magnetic resonance (NMR)

A 20% alcohol solution was prepared using deuterium oxide, D_2O

(99.9% atom D, containing 0.75% weight trimethylsilyl propionate [TSP]; Sigma Aldrich), in a 5 mm NMR tube. Spectra were acquired on a Varian Mercury 400 spectrophotometer (Varian Associates Inc, Palo Alto). The spectrophotometer consisted of a 54 mm bore size unshielded Oxford Magnet (Oxford Instruments Ltd, Oxfordshire) operating at a proton frequency of 400 MHz connected to a Varian mercury VX console with a performa 1 pulsed field gradient amplifier (20 gauss per centimeter) and run on Varian 6.1B software. A standard ^{1}H NMR spectrum was recorded at room temperature using a quantitative ^{1}H NMR pulse sequence. Standard 1 D processing with additional zero-filling to 64 k was applied and integrals measured. The integrals from the methyl signals of ethanol and methanol were used for comparison and quantification (Berger and Braun, 2004).

Toxic and genotoxic effects of beer to mouse bone marrow cells using the micronucleus assay

About 7 to 8 week-old male inbred NIH mice were purchased from the University of Free State, Proefdiereenhied animal unit (Bloemfontein, Republic of South Africa) and used in the experiment after 1 week of acclimatization. All the animals were allowed free access to pelleted horse feeds (Voernet (PTY) LTD Republic of South Africa) and tap water *ad libitum* through out the 1-week acclimatization period. Three groups of five animals per group were given filtered sorghum beer (100, 50 and 25%, respectively), diluted with tap water to the desired concentration as the only liquid source for 35 days. Both negative and positive control groups received water *ad libitum* during the 35 days period. On the 34th day, the positive control group animals were given the indirect-acting mutagen, cyclophosphamide monohydrate (CP) (Fluka Biochemika, Germany) intraperitoneally in a single dosing regimen at the volume of 10 ml/kg of body weight at the concentration of 40 mg/kg (Salvadori et al., 1992; Gimmler et al., 1999) dissolved in purified water BP (Medicolab, Republic of South Africa).

Slide preparations of the bone marrow smears were according to the method of Asita et al. (2008). Two smears were prepared for each animal. Slides were coded and scored blind using the OLYMPUS CXS21 light microscope.

To evaluate bone marrow toxicity, the ratio PCE/ (PCE + NCE) was calculated by counting a total of 1000 erythrocytes per animal using these slides. All together 2000 polychromatic erythrocytes per animal were examined for the presence of micronuclei for each individual animal, which means 10,000 PCEs scored per dose group, to determine the occurrence of MNPCEs. Frequency of MNPCEs per 1000 = (MNPCEs/ total number of PCEs counted) ×1000.

Statistical analysis

Data of the concentration of metal ions in the beer and water samples were expressed as mean ± S.D. The data were subjected to the statistical analysis by ANOVA (one-way), to compare for difference in the metal ions concentrations in the beer samples from one source collected on three different sampling days; to compare for difference in the concentrations of each metal ion in the beer and water samples from the four different sources; to compare for difference in the alcohol content in the beers from the four different sources. $P < 0.05$ was considered as the level of significance.

Student's t-test was used to compare the concentration of each metal ion in the beer from each source and its concentration in the water that was used for brewing. $P < 0.05$ was considered as the level of significance.

To evaluate bone marrow toxicity only the beer sample from the Sea Point site was used because of its location in the city.

Statistical differences between each concentration group and the concurrent negative control in the incidences of MNPCE/1000 PCEs and the PCE/ (PCE + NCE) ratio were determined by the Mann-Whitney U test for 2 independent samples. $P < 0.05$ was considered as the level of significance. The statistical analyses were performed using the SPSS 10.0 statistical program.

RESULTS

Metal ions concentrations

Metal ions in the beer and the corresponding water samples

The concentrations (mg/L) of the metal ions in the beer and the corresponding water samples used to brew the beers were as follows: As (0.012 – 0.059 and 0.033 – 0.383 respectively), Cd (0.032 – 0.035 and 0.023 – 0.260 respectively), Co (0.127 – 0.160 and 0.354 – 0.384 respectively, Cr (0.052 – 0.069 and 0.033 – 0.383 respectively), Cu (0.004 – 0.057 and 0.349 – 0.400 respectively), Fe (0.070 – 0.600 and 0.342 – 4.054 respectively), Hg (0.131 – 0.150 and 0.021 – 0.381 respectively), Ni (0.004 – 0.031 and 0.135 – 0.384 respectively), Pb (0.019 – 0.029 and 0.332 – 0.400 respectively), Se (0.645 – 0.942 and 0.371 – 0.420 respectively) and Zn (0.317 – 6.337 and 3.743 – 4.019 respectively) (Table 1).

1) The mean concentrations of the metal ions in the beer samples of the three sampling days did not differ significantly ($p > 0.05$) for the beers from Mafefooane, Mahlanyeng and Sea Point.
2) Only the mean concentrations of Cu ions in the beer samples of the three sampling days from Mafikeng differed significantly ($p< 0.05$).
3) The mean concentrations of As, Cr, Fe, Ni Co, Cu, Pb, Zn and Se in the beer samples from the four sources were significantly different ($p< 0.05$).
4) The mean concentrations of Cd and Hg in the beer samples from the four sources were not significantly different ($p> 0.05$).

Metal ions in the water samples

A Comparison of the concentrations of individual metal ion in the water samples from the four sources is presented in Table 2.
1) The concentrations of As, Cd, Cr, Fe, Hg, Ni and Se in the water samples from the four sources were significantly different ($p< 0.05$).
2) The concentrations of Co, Cu, Pb and Zn in the water samples from the four different sources were not significantly different ($p> 0.05$).
3) The mean concentrations of As, Co, Cr, Cu, Fe, Hg, Ni, and Zn in the water samples from Mahlanyeng were higher than their mean concentrations in the water

Table 1. Concentration of metals in the beers and waters (mg/L).

Metal	Beer/ water	Mafefooane metal concentration (mg/L)		Mahlanyeng metal concentration (mg/L)		Sea point metal concentration (mg/L)		Mafikeng metal concentration (Mg/L)	
		Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
As*	Beer	0.038	0.001	0.013	0.001	0.059‡	0	0.054‡	0.003
	Water	0.377	0.011	0.383	0.049	0.037	0.01	0.033	0.01
Cd	Beer	0.036	0.001	0.036	0.001	0.033‡	0.001	0.034‡	0.002
	Water	0.26	0.004	0.211	0.035	0.024	0.017	0.023	0.005
Co*	Beer	0.161	0.002	0.132	0.001	0.145	0.016	0.142	0.003
	Water	0.378	0.008	0.384	0.018	0.355	0.049	0.364	0.087
Cr*	Beer	0.075	0.007	0.053	0.001	0.066	0.001	0.061	0.003
	Water	0.385	0.015	0.391	0.02	0.364	0.001	0.351	0.004
Cu*	Beer	0.007	0.005	0.057	0	0.011	0.002	0.025†	0.003
	Water	0.395	0.001	0.4	0.036	0.392	0.005	0.349	0.1
Fe*	Beer	0.605	0.005	0.381	0.001	0.591	0.002	0.071	0.001
	Water	3.929	0.008	4.054	0.072	3.59	0.2	0.342	0.1
Hg	Beer	0.147	0.003	0.151	0.001	0.149‡	0.001	0.141	0.01
	Water	0.21	0.013	0.381	0.001	0.022	0.003	0.201	0.011
Ni*	Beer	0.004	0.001	0.01	0.001	0.032	0.001	0.028	0.002
	Water	0.381	0.065	0.384	0.01	0.351	0.02	0.135	0.042
Pb*	Beer	0.031	0.002	0.02	0.001	0.022	0.001	0.027	0.002
	Water	0.373	0.003	0.399	0.009	0.4	0.011	0.332	0.1
Se*	Beer	0.646‡	0.001	0.94‡	0.001	0.766‡	0.002	0.776‡	0.016
	Water	0.42	0.01	0.409	0.008	0.371	0	0.407	0.003
Zn*	Beer	0.318	0.001	0.773	0.019	0.775	0.002	6.345‡	0.009
	Water	3.746	0.1	4.019	0.176	3.916	0.224	3.743	0.764

n = sample size (3); S.D = Standard deviation * = statistically significant difference in metal ions concentrations in the beers from the different sources**;** † = statistically significant difference in metal ions concentrations in the beers of the three sampling days from one source; ‡ = statistically higher metal ion concentration in beer sample than in the water used for brewing.

samples from Mafefooane, Sea Point and Mafekeng. The mean mean concentration of Pb in the water sample from Mahlanyeng was the same as that from Sea Point 4). The concentrations of Cd and Se in the water samples from Mafefooane were higher than their concentrations

Table 2. Comparison of the concentrations of individual metal ions in the water from the four different sources.

Water source	Sample	Metal ions concentration in beer samples (mg/L)										
		As	Cd	Co	Cr	Cu	Fe	Hg	Ni	Pb	Se	Zn
	a	0.387	0.264	0.381	0.382	0.395	3.936	0.195	0.309	0.376	0.430	3.746
Mafefooane (bore hole)	b	0.365	0.256	0.369	0.372	0.395	3.929	0.218	0.399	0.370	0.420	3.646
	c	0.380	0.260	0.383	0.402	0.396	3.921	0.216	0.436	0.373	0.410	3.846
	a	0.328	0.250	0.384	0.411	0.364	4.054	0.381	0.394	0.390	0.410	4.149
Mahlanyeng (tap water)	b	0.422	0.181	0.367	0.372	0.436	3.982	0.382	0.384	0.408	0.416	4.089
	c	0.398	0.202	0.402	0.391	0.400	4.126	0.380	0.374	0.400	0.401	3.819
	a	0.047	0.044	0.376	0.363	0.398	3.790	0.021	0.351	0.404	0.371	3.688
Sea Point (tap water)	b	0.037	0.014	0.389	0.364	0.390	3.590	0.019	0.331	0.408	0.371	4.135
	c	0.027	0.014	0.299	0.365	0.389	3.390	0.025	0.371	0.388	0.371	3.926
	a	0.033	0.023	0.465	0.351	0.349	0.342	0.206	0.112	0.232	0.410	3.076
Mafikeng (bore hole)	b	0.023	0.028	0.311	0.347	0.449	0.242	0.189	0.110	0.332	0.407	4.576
	c	0.043	0.018	0.317	0.355	0.249	0.442	0.209	0.184	0.432	0.404	3.576
One-way ANOVA result	Test $_{stat}$	175.607*	115.942*	0.206	6.68*	0.593	679.546*	894.288*	26.114*	1.202	32.741*	0.327

* = Statistically significant difference in metal ion concentrations in the waters from the four different sources.

in the water samples from Mahlanyeng, Sea Point or Mafekeng. And the water samples from the Mafefooane site contained higher concentrations of As, Co, Cr, Cu, Fe, Hg and Ni than the water samples from Sea Point and Mafekeng.

5) The water samples from the Sea point site contained higher concentrations of As, Cd, Cr, Cu, Fe, Hg, Ni, Pb and Zn than the water samples from the Mafekeng site. However, water from the Mafekeng site contained higher concentrations of Co and Se than the water samples from the Sea Point site.

According to the number of metal ions with the highest concentration, the water samples from the four sites were ranked as follows: Mahlanyeng > Mafefooane > Sea Point > Mafekeng.

Comparison of metal ions concentrations in beer and the water used for brewing

The results are presented in Table 3 and summarized as follows:

1) The concentrations of Co, Cr, Cu, Fe, Ni and Pb in the beer samples were significantly lower ($p< 0.05$) than their concentrations in the waters from the 4 water sources. The observation suggested that the brewing process resulted in a lowering of the concentrations of these metal ions in the beers.

2) The concentration of Se ions in the beers from Mafefooane and Mahlanyeng, As, Cd, Hg and Se ions in the beers from Sea Point and As, Cd, Se and Zn ions in the beers from Mafikeng, were significantly higher ($p< 0.05$), than their concentrations in the waters used for brewing. The observation suggested that these metal ions were introduced into the beers from extraneous sources during the brewing process.

Comparison of metal ions in the beer and water samples with World Health Organization (WHO, 2008) guideline values for drinking water

The concentrations of Hg, Pb and Se in the water and beer samples were higher than the WHO values. The concentrations of Cu in all the water and beer samples were lower than the WHO value. With the exception of beer samples from

Table 3. Comparison of the concentration of each metal ion in the beer with their concentration in the water for each source (t-values).

Metal	Beer and water source			
	Mafefooane	Mahlanyeng	Sea point	Mafikeng
As	52.274 ↓	13.107 ↓	- 3.811 ↑	- 3.516↑
Cd	96.143 ↓	8.585 ↓	-0.931	- 3.755 ↑
Co	48.509 ↓	24.884 ↓	7.116↓	4.418↓
Cr	31.726 ↓	30.009 ↓	446.5↓	99.681↓
Cu	135.312 ↓	16.503 ↓	127.791↓	5.61↓
Fe	648.992 ↓	88.364 ↓	25.968↓	4.7↓
Hg	8.292 ↓	345.5 ↓	-67.352 ↑	7.309↓
Ni	9.997 ↓	64.729↓	27.644↓	4.406↓
Pb	162.383 ↓	72.2 ↓	61.827↓	5.282↓
Se	-39.022 ↑	-122.075 ↑	-342.08 ↑	- 38.195 ↑
Zn	59.379 ↓	31.805 ↓	24.329 ↓	- 5.901↑

↓ = Metal ion in beer sample was significantly lower than the concentration in the water used for brewing). ↑ = Metal ion in beer sample was statistically significantly higher than the concentration in the water used for brewing.

Mahlanyeng and water samples from Sea Point, the concentrations of As, Cd and Cr in the water and beer samples were higher than the WHO values. The concentrations of Ni in the water samples were higher than, and their concentrations in the beer samples were lower than, the WHO guideline values for drinking water. No guideline values are available for Co, Fe and Zn (Table 4).

Alcohol concentrations in one day samples (n = 5) of beer from the four sources

Only the total alcohol (determined by Back titration method), methanol and ethanol (determined by NMR) concentrations (% alcohol by volume) could be detected in the methods used.

1) The mean total alcohol, methanol and ethanol concentrations in the beers from the four sources were as follows: Mafefooane (6.36, 0.2, and 6.4 respectively); Mahlanyeng (5.30, 0.18, and 5.30 respectively); Sea Point (7.42, 0.08 and 7.4 respectively) and Mafikeng (7.42, 0.2 and 6.4 respectively). The concentration of ethanol was higher than that of methanol in all the beer samples.
2) The mean total alcohol, methanol and ethanol concentrations in the beer samples from the four sources were statistically different ($p< 0.05$) (Table 5).

Mice bone marrow erythropoietic cell toxicity and clastogenicity (micronucleus assay)

The results obtained in the micronucleus test of the effect of indigenous sorghum beer on the incidence of MNPCE, MNNCE and frequency of PCEs in total erythrocytes are summarized in Table 6, for the beer sample from the Sea point source only. No increases in the frequency of MNPCE were observed in any of the groups of mice that received sorghum beer for 35 days. However, only in the group that received 50% beer was any statistically significant decrease in the PCE/NCE ratio observed (Table 6).

DISCUSSION

As presented in Table 1, locally brewed sorghum beer from Mafefooane, Mahlanyeng, Sea Point and Mafikeng sources in Lesotho contained significantly different ($p< 0.05$) concentrations of As, Cr, Fe, Ni Co, Cu, Pb, Zn and Se in the ranges of 0.004 mg/L for Cu and Ni to 0.942 and 6.337 mg/L for Se and Zn respectively. The concentrations of Cd and Hg in the beer samples however, were not significantly different ($p> 0.05$). No significant differences ($p > 0.05$) in the mean metal ions concentrations in the beer samples of the three sampling days for the samples from Mafefooane, Mahlanyeng and Sea Point. Wide variations in the levels of metals were thus detected in the beers. In a study of the concentrations of metal ions in wine samples from the province of Mendoza in Argentina, the concentrations of aluminium, cadmium, calcium, copper, iron, lead, zinc, and chromium were between 0.017-0.018 µg/L, 0.001-0.0047 µg/L, 0.010 - 0.015 µg/L, 0.023-0.028 µg/L, 0.480-0.790 µg/L, 0.050-0.090 µg/L, 0.024-0.130 µg/L, and <0.0002-0.00625 µg/L, respectively (Lara et al., 2005). In another study, different concentrations and diversity of metals were found in beverages; highest for red wine samples (30 metals totaling 5620.54 ± 123.86 ppb, that is, µg/L) followed by apple juice (15 metals totaling 1339.87

Table 4. Comparison of metal ions in beer and water with WHO guideline values for drinking water.

Metal	WHO guideline value (mg/L)	Metal ions concentration (mg/L) 𝔛 ± S.D (n=3)							
		Mafefooane (bore hole)		Mahlanyeng (tap water)		Sea Point (tap water)		Mafikeng (bore hole)	
		Water	Beer	Water	Beer	Water	Beer	Water	Beer
As	0.01 mg/L (provisional) 10 µ /L	0.377±0.011*	0.038±0.002*	0.383±0.049*	0.013±0.003	0.037±0.010*	0.059±0.003*	0.033±0.010*	0.052±0.004*
Cd	0.003 mg/L	0.260±0.004*	0.036±0.006*	0.211±0.035*	0.036±0.006*	0.024±0.017	0.034±0.005*	0.023±0.005*	0.036±0.004*
Co	No limit listed	0.378±0.008	0.161±0.002	0.384±0.017	0.133±0.007	0.354±0.049	0.150±0.044	0.365±0.087	0.138±0.010
Cr	0.05 mg/L provisional	0.385±0.015*	0.072±0.007*	0.391±0.020*	0.053±0.003	0.364±0.001*	0.066±0.006*	0.351±0.004*	0.058±0.002*
Cu	2 mg/L	0.395±0.001	0.004±0.001	0.400±0.036	0.057±0.001	0.392±0.005	0.010±0.004	0.349±0.100	0.022±0.003
Fe	No limit listed 1–3 mg/L acceptable	3.929±0.007*	0.607±0.091	4.054±0.072*	0.381±0.000	3.590±0.200*	0.589±0.010	0.342±0.100	0.070±0.001
Hg	0.006 mg/L	0.210±0.013*	0.147±0.007*	0.381±0.001*	0.151±0.000*	0.021±0.003*	0.148±0.001*	0.201±0.011*	0.131±0.044*
Ni	0.07 mg/L	0.381±0.065*	0.004±0.001	0.384±0.010*	0.010±0.000	0.351±0.020*	0.032±0.005	0.135±0.042*	0.026±0.004
Pb	0.01 mg/L	0.373±0.003*	0.030±0.000*	0.400±0.009*	0.021±0.001*	0.400±0.011*	0.021±0.002*	0.332±0.100*	0.025±0.005*
Se	0.01 mg/L	0.420±0.010*	0.646±0.051*	0.409±0.007*	0.943±0.039*	0.371±0.000*	0.766±0.001*	0.407±0.003*	0.757±0.050*
Zn	No limit listed >3 mg/L unacceptable	3.746±0.100*	0.318±0.021	4.019±0.176*	0.773±0.003	3.916±0.224*	0.775±0.006	3.743±0.764	6.343±0.018*

*Concentration ($\bar{x}$ ± S.D.) higher than the WHO guideline values for drinking water. WHO (2008).

± 10.84 ppb) and stout (14 metals totaling 464.85 ± 46.74 ppb) (Hague et al., 2008).

The levels of 0.004 mg/L for Cu and Ni to 0.942 and 6.337 mg/L for Se and Zn, respectively, are equivalent to 0. 4 µg/L for Cu and Ni to 942.00 µg/L and 6337.00 µg/L for Se and Zn, respectively. Thus the metal ions concentrations in the sorghum beers in the present study were between 400 and 100000 times their concentrations in the wines and beverages in the two studies cited above. The concentration of, 6337.00 µg/L for zinc in the present study, for instance, was 48746.2 times the concentration of 0.130 µg/L for zinc in the Argentinean wines. Diets are known to account for the largest exposure to metals and metals play widespread roles in health and disease which range from the requirement for intake of essential trace elements to toxicity associated with metal overload (Hague et al., 2008). Many of the toxic effects associated with metals are still under investigation, especially for low concentrations and for lifetime exposure, therefore the upper safe limits for many metal ions are unavailable (Lagerkvist and Oskarsson, 2007). However, the World Health Organization (WHO, 2008), has provided guideline values (mg/L) of the concentrations of some metals in drinking water. The concentrations of Co, Hg, Pb and Se in the beer samples were stronger than the WHO guideline values for drinking water while the concentrations of Ni and Cu were lower than the WHO guideline values. The concentrations of As and Cr in the beers were higher than their WHO guideline values for beers from three sources but not the Mahlanyeng source. No WHO guideline values was available for concentrations of Co, Fe and Zn. Many metal ions are associated with enhanced oxidative stress, inflammation and cancer (U.S. EPA, 1989; Valko et al., 2006). Epidemiological evidence indicates that As is associated with cancers of the skin and internal organs, as well as with vascular disease (Luster and Simeonova, 2004).

Symptoms of short-term exposures of animals to cadmium include muscle cramps, sensory disturbances, liver injury, convulsions, shock and kidney failure (Rogers, 1996). The addition of cobalt salts as foam improvers in beer in the 1950s led to deaths in North America (Priest and Stewart, 2006). Chromium toxicity is very dependent on the species and oxidation states present and is still subject to considerable debate (Fisher and Naughton, 2005; Seenivasan et al., 2008). Some reported effects of high doses of copper include decreased levels of superoxide dismutase, a key enzyme for protection against oxidative damage which may be enhanced by

Table 5. Comparison of the alcohol content of the beers (% by volume).

Beer source	Total alcohol		Methanol		Ethanol	
	Mean	S.D	Mean	S.D	Mean	S.D
Mafefooane	6.36	0.666	0.2	0.021	6.4	0.37
Mahlanyeng	5.5	0.566	0.18	0.055	5.3	0.472
Sea Point	7.42	0.634	0.08	0.016	7.4	0.437
Mafikeng	7.42	0.634	0.2	0.019	6.4	0.337
One-way ANOVA result	13.146*		16.296*		22.15*	

n = sample size (5); * = statistically significant difference in alcohol concentrations in the beers from the different sources.

Table 6. Summary table of the incidence of MNPCE and frequency of PCEs in total erythrocytes in mouse bone marrow of 8-week-old males inbred NIH mice after 35 days of sorghum beer *ad libitumin* or water as negative control.

Treatment (% alcohol)	PCE (Data of five animals per group)				NCE scored of 5000 erythrocytes/group	PCE/ (PCE + NCE) (individual and Mean ±S.D)
	PCE scored	MNPCE (individual and total)	MNPCE/ 1000 PCE			
			Individual data	Group Mean ± S.D		
0	10000	3; 2; 2; 3; 4; (14)	1.5; 1;1;1.5; 2	1.40 ± 0.40	1966	0.54; 0.75; 0.75; 0.48; 0.52; (0.61 ± 0.13)
25	10000	6; 2; 2; 0; 4; (14)	3; 1; 1; 0; 2	2.8 ± 1.14	2176	0.54; 0.61; 0.53; 0.63; 0.52; (0.57 ± 0.05)
50 (†)	10000	4; 5; 2; 4; 0; (15)	2; 2.5; 1; 2; 0	3.0 ± 1.0	2829	0.48; 0.43; 0.46; 0.31; 0.49; (0.43 ± 0.07)
100	10000	0; 2; 2; 0; 2; (6)	0; 1; 1; 0; 1	0.60 ± 0.55	2621	0.42; 0.49; 0.43; 0.52; 0.52; (0.48 ± 0.05)
CP († and *)	10000	7; 15; 10; 8; 23 (63)	3.5; 7.5; 5; 4; 11.5	6.3 ±3.29	2888	0.36; 0.47; 0.37; 0.49; 0.42; (0.422± 0.058)

PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocyte; MNPCE = Micronucleated PCE; CP = Cyclophosphamide; * = Clastogenic; † =Toxic.

labile copper, which may initiate or exacerbate inflammatory disorders (Valko et al., 2006). High levels of ingested iron causes impaired oxidative phosphorylation and mitochondrial dysfunction particularly in the liver, which can result in cell death (Zacharski et al., 2004). Mercury toxicity is very dependent on the species and oxidation states present and is still subject to considerable debate (Fisher and Naughton, 2005; Seenivasan et al., 2008). Nickel has been shown to cause tissue injury and genotoxicity, to be cytotoxic to T-lymphocyte cells and to cause DNA damage (Sunderman et al., 1976; environmental protection agency (EPA), 1995**;** Angela et al. 2006). The primary affects of lead are on the peripheral and central nervous system, kidney function, blood cells, and the metabolism of vitamin D and calcium. Lead can also cause hypertension, reproductive toxicity, and developmental effects (ATSDR, 1992).

Health effects of Selenium are very much dependent on dose. High blood levels of selenium is known to cause selenosis with symptoms that include gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue, irritability, and mild nerve damage (Goldhaber, 2003).

Zinc has numerous reported beneficial and detrimental effects, being essential components of many enzymes (Cherny et al., 2001; Alessio et al., 2007). Too little zinc can cause slow wound healing and skin sores, decreased sense of taste and smell, loss of appetite and damage in immune system. According to the Agency for toxic substances and disease registry (ATSDR) however, exposure to large amount of zinc for a long time can cause anemia, pancreas damage and low levels of high density lipoprotein cholesterol (ATSDR, 2005).

The results of the present study (Table 1) showed that the water samples from the four sources contained significantly different ($p< 0.05$) concentrations of As, Cd, Cr, Fe, Hg, Ni and Se but not significantly different concentrations of Co, Cu, Pb and Zn. The concentrations (mg/L) of the metal ions were in the ranges: As (0.033 – 0.383), Cd (0.023 – 0.260), Co (0.354 – 0.384, Cr (0.033 – 0.383), Cu (0.349 – 0.400), Fe (0.342 – 4.054), Hg (0.021 – 0.381), Ni (0.135 – 0.384), Pb (0.332 – 0.400), Se (0.371 – 0.420) and Zn (3.743 – 4.019).

According to the number of metal ions with the highest concentration, the water samples were ranked as follows: Mahlanyeng > Mafefooane > Sea Point > Mafekeng.

In a study of the presence of some metal ions in drinking water samples in Turkey, the metal ions concentrations varied as follows; Cu 0.17-1.19 µg/L, Fe 16.11-79.30 µg/L, Pb 0.18-0.99 µg/L and Mn 0.15-2.56 µg/L (Soylak et al., 2002).

Another study in Ghana of metal ions in drinking water demonstrated the presence of metal ions (mg/L) and the variation of their concentrations in water from different sources for Cu (1.19-2.75), Fe (0.05-0.85), Zn (0.04-0.15), Mn (0.003-0.011) and Al (0.05-0.15) (Akoto and Adiyiah, 2007). Whereas the concentrations of Cu in the present study were lower than their concentrations in the waters in the Ghana study, the concentrations of Zn were higher than their concentrations in the waters in the Ghana study. The concentrations of Fe in the waters in the present study were in the same ranges as their concentrations in the waters in the Ghana study. The concentrations of Cu, Zn and Fe in the waters of the present study and the waters in the Ghana study were much higher (>2000x), than their concentrations in the waters in the Turkey study. These observations could be attributed to differences in geology of the different regions. Furthermore, the concentrations of As, Cr, Hg, Ni, Pb and Se in the water samples analyzed in the present study were higher than the WHO guideline values for drinking water. The concentrations of Cd were also higher than the WHO guideline values in the waters from Mafefooane, Mahlanyeng and Mafikeng while the concentrations of Cu were lower than the WHO guideline values. The sources of the water at the 4 sites were as follows, Mafefooane (Bore hole), Mafikeng (Bore hole), Mahlanyeng (Tap water), Sea Point (Tap water).

The concentrations of Co, Cr, Cu, Fe, Ni and Pb in the beer samples were significantly lower ($p< 0.05$) than their concentrations in the water from each of the 4 water sources which suggested that the brewing process resulted in a lowering of the concentrations of these metal ions in the beers. However, the concentrations of Se in the beers from Mafefooane and Mahlanyeng, As, Cd, Hg and Se ions in the beers from Sea Point and As, Cd, Se and Zn ions in the beers from Mafikeng, were significantly higher ($p< 0.05$), than their concentrations in the waters used for brewing. The observations suggested that these metal ions were introduced into the beers from extraneous sources during the brewing process. The extraneous source(s) of the metal ions in the beer samples in the cases where their concentrations were higher than their concentrations in the water used for brewing was not certain. One possible extraneous source of the metal ions could be the metal drums in which the beers were brewed. However, according to Larsson et al. (1998), as competition between brewers for customers set in, brewers invented brewing techniques that produced beer at lower and more affordable prices to consumers. Some of the new techniques, it is claimed, involved the introduction of radio batteries, brake fluid and car battery acids into the beer to make it stronger (that is, more intoxicating). Batteries are made of many different metal elements/compounds and, depending on the brand, may contain aluminum (Al), copper (Cu), mercury (Hg), arsenic (As), zinc (Zn), lead (Pb), nickel (Ni) and cadmium (Cd) (Dhar, 1973).

The beer samples contained measurable concentrations (percentage of alcohol by volume) of ethanol and methanol only, whose concentrations in the beers from the different sources varied significantly and with higher concentrations of ethanol than methanol in the beers from all the sources. The concentrations (percentage of alcohol by volume) of total alcohol in the beer samples were between 5.30 and 7.42, equivalent to 53,000 to 74,200 mg/L. The concentrations (in percentage of alcohol by volume) of methanol ranged from 0.08 to 0.2, equivalent to 800 to 2000 mg/L and for ethanol, 5.30 to 7.4, equivalent to 53,000 to 74,000 mg/L. The total alcohol contents in the beers from the different sources were thus different, which suggested that different brewers employed different methods. The average concentration of 6.36% alcohol in the beers was different from a reported concentration of 3% for *Joala*, another traditional beer that is brewed and consumed in Lesotho (Turner, 2001).

The United States Environmental Protection Agency, in their multimedia environmental goals for environmental assessment recommends a minimum acute toxicity concentration of methanol in drinking water at 3.9 ppm (3.9 mg/L), with a recommended limit of consumption below 7.8 mg/day (Cleland and Kingsbury, 1977). Death from consumption of the equivalent of 6 g of methanol has been reported the U.S. Department of Health, Education, and Welfare (HEW) (HEW, 1976; Wimer et al., 1983). The 1400 mg/L average content of methanol in the beers is equivalent to 1.4 mg/ml. Given that the daily volumes of traditional beer that were consumed on a typical drinking day ranged from 340 to 10 000 ml with a mean (SD) of 2634 (± 1737 ml) (Siegfried, 2001), a drinker who consumed the minimum of 340 ml/day, would have ingested 476 mg of methanol, which far exceeds the recommended limit of consumption of below 7.8 mg/day. For an average adult, the fatal ingested dose is approximately 1 L (approximately 2 pints) of 40 to 55% ethanol (the percentage found in whiskey, gin, rum, vodka, or brandy) consumed within a few minutes (Gosselin, 1984). In the present study, the beers contained on average, 6.36% total alcohol, equivalent to 36.125 g alcohol/pint (568 ml), made up of about 99% ethanol. Western beers contain, on average, 5% alcohol (Turner, 2001).

In the present study, the beer samples did not induce chromosomal aberrations in mice bone marrow but induced erythropoietic cell toxicity (decrease in the PCE/NCE ratio) at the 50% concentration of beer only. In one reported study in which rodents were exposed to ethanol *in vivo*, the ethanol did not induce micronuclei in mice, but conflicting results were obtained in rats. Ethanol induced sister chromatid exchanges in mouse embryos exposed *in vivo* and, in another study, chromosomal aberrations in rat embryos exposed *in vivo* (IARC, 1988). Statistically significant sister chromatid exchanges were observed in the bone marrow of male mice when 10 or 20% ethanol (approximately 20000 or 40000 mg/kg/day) was given as the only liquid source for 3 to 16 weeks (Obe et al., 1979). In the present study however, the mean alcohol concentration of the beers was much lower (6.36%) than, and the methods of administration of the beers different from, those of the studies previously cited.

Conclusions

The results showed that the beers from the four different sources contained significantly different ($p< 0.05$) concentrations (mg/L) of As, Cr, Fe, Ni Co, Cu, Pb, Zn and Se but similar ($p> 0.05$) concentrations of Cd and Hg. The concentrations (mg/L) of total alcohol (53,000 to 74,200); methanol (800 to 2000) and ethanol (53,000 to 74,000) differed significantly ($p < 0.05$) which suggested different brewing methods. Concentrations of Se, As, Cd, Hg, Se As, Cd, Se and Zn in the beers were higher ($p< 0.05$), than in the brewing water which suggested that these metals were introduced from extraneous sources.

Beer from only one source was assessed for genotoxicity. It was not clastogenic but induced erythropoietic cell toxicity at the 50% concentration only ($p < 0.05$).

The high alcohol and metal ions concentrations in the beers and water have implications for public health and highlights the need for public awareness, regulation and standardization of production methods and consumption.

Further research is necessary to ascertain the source(s) of the metal ions.

ACKNOWLEDGEMENT

Authors would like to express their appreciation to Mr. Andreas Thakaso for his help with the animal husbandry during this work.

REFERENCES

Aísa R, Pueyo F (2004). Endogenous longevity, health and economic growth: a slow growth for a longer life? Economics Bulletin, 9(3): 1-10.http://www.economicsbulletin.com/2004/volume9/EB–03I10002A.pdf.

Akoto O, Adiyiah J (2007). Chemical analysis of drinking water from some communities in the Brong Ahafo region. Int. J. Environ. Sci. Technol., 4 (2): 211-214.

Alessio L, Campagna M, Lucchini R (2007). From lead to manganese through mercury: mythology, science, and lessons for prevention. Am. J. Ind. Med., 50: 779-787.

Ang HH, Lee KL, Kiyoshi M (2005). Determination of lead in Smilax luzonensis herbal preparations in Malaysia. Int. J. Toxicol., 24: 165-171.

Angela AU, Jinny HA, Hernandez M, Polotsky A, Hungerford DS, Frondoza CG (2006). Nickel and vanadium metal ions induce apoptosis of T-lymphocyte Jurkat cells. J. Biomed. Mater. Res. Pt A*,* 79(3): 512-521.

AOAC. Association of Official Analytical Chemists (1995). Official Methods of Analysis. 16th Edition. Washington DC, 28: 2-3.

Asita, AO, Dingann ME, Magama S (2008). Lack of modulatory effect of asparagus, tomato, and grape juice on cyclophosphamide-induced genotoxicity in mice. Afr. J. Biotechnol., 7 (18): 3383-3388.

ATSDR. Agency for Toxic Substance and Disease Registry (1992). Case Studies in Environmental Medicine: Lead Toxicity.

ATSDR. Agency for Toxic Substances and Disease Registry (2005). Toxicological Profile for Zinc (Update), Atlanta, GA: U.S. Department of Public Health and Human Services, Public Health Service.

Berger S, Braun S (2004). 200 and More NMR Experiments. Second Edition, Wiley-VCH, Germany, pp. 315-317.

Bonner P (1990). "Desirable or Undesirable Women? Liquor, Prostitution, and the Migration of Basotho Women to the Rand, 1920-1945," In: Cherryl Walker (Ed). Women and Gender in Southern Africa to 1945. Cape Town: David Philip; London: J. Currey.

Bourgeault-Tassé I (2011). Taking stock: Alternative indicators of national well-being. http://www.researchsea.com/html/article.php/aid/5840/cid/6/research/taking_stock__alternative_indicators_of_national__wellbeing.html?PHPSESSID=fg9e65e3qp443iair1f79s8ds1. Accessed on 2011 07-10

Cherny RA, Atwood CS, Xilinas ME, Gray DN, Jones WD, McLean CA, Barnham KJ, Volitakis I, Fraser FW, Kim YS, Huang XD, Goldstein LE, Moir RD, Lim JT, Beyreuther K, Zheng H, Tanzi RE, Masters CL, Bush AI (2001). Treatment with a copper-zinc chelator markedly and rapidly inhibits β-amyloid accumulation in Alzheimer's disease transgenic mice. Neurology, 30: 665-676.

Cleland JG, Kingsbury GL (1977). Multimedia Environmental Goals For Environmental Assessment. U.S. Environmental Protection Agency: EPA-600/7-77-136b, E-28, November.

Dhar SK (1973). Metal Ions in Biological Systems: Studies of Some Biochemical and Environmental Problems In: Sanat K, Dhar (Editor) Plenum Press.

Fisher A, Naughton DP (2005). Therapeutic chelators for the twenty first Century: New treatments for iron and copper mediated inflammatory and neurological disorders. Curr. Drug. Deliv. 2: 261-

268.
Gimmler-Luz MC, Cardoso VV, Sardiglia CU, Widholzer DD (1999) Transplacental inhibitory effect of carrot juice on the clastogenicity of cyclophosphamide in mice. Genet. Mol. Biol., 22(1): 1-13.
Goldhaber SB (2003). Trace element risk assessment: essentiality vs. toxicity. Regulatory Toxicology and Pharmacology. 38: 232-42.
Gosselin RE (1984). Ethyl alcohol. In: Gosselin, RE, Smith RP, Hodge HC (Eds). Clinical Toxicol. Commercial Products. 5th ed. Baltimore: Williams and Wilkins, pp. III-267.
Hague T, Petroczi A, Andrews PLR, Barker J, Naughton DP (2008). Determination of metal ion content of beverages and estimation of target hazard quotients: a comparative study. Chem. Cent. J., 2: 13.
Halliwell B, Gutteridge JM (2007). Editors. Free radicals in biology and medicine. 4. Oxford University Press, UK.
HEW. US Department of Health, Education, and Welfare (1976). Occupational Exposure to Methyl Alcohol, HEW Pub. No. (NIOSH) (March), pp. 76-148. http://web.deu.edu.tr/geomed2010/2007/Sandal.pdf - Accessed on July 14, 2011.
IARC. International Agency for Research on Cancer (1988). Monographs on the evaluation of carcinogenic risks to humans. Vol. 44. Alcohol drinking. World Health Organization. 1988: 35.
Lagerkvist B, Oskarsson A (2007). Vanadium. Handbook on the Toxicology of Metals. Third Edition, pp. 905-923.
Lara R, Cerutti S, Salonia JA, Olsina RA, Martinez LD (2005). Trace element determination of Argentine wines using ETAAS and USN-ICP-OES. Food Chem. Toxicol., 43(2): 293-297.
Larsson A, Mapetla M, Schlyter A (1998). Changing Gender Relations in Southern Africa: Issues of Urban Life. The Nordic Africa Institute. 99911-31-21-3.
Luster MI, Simeonova PP (2004). Arsenic and urinary bladder cell proliferation. Toxicol. Appl. Pharmacol., 198: 419-423.
Navarro-Blasco I, Alvarez-Galindo JI (2005). Lead levels in retail samples of Spanish infant formulae and their contribution to dietary intake of infants. Food Addit. Contam., 22: 726-734.
Obe G, Natarajan AT, Meyers M, Hertog AD (1979). Induction of chromosomal aberrations in peripheral lymphocytes of human blood *in vitro*, and of SCEs in bone-marrow cells of mice *in vivo* by ethanol and its metabolite acetaldehyde. Mutat. Res. 68: 291-294.
OECD (2006). Life Expectancy vs. Alcohol Consumption by Country. Organisation for Economic Co-operation and Development: Health Data.
Priest FG, Stewart GG (2006). Handbook of Brewing (Editors: 2nd Edition) Taylor & Francis, Publishers.
Rogers JM (1996). The developmental toxicity of cadmium and arsenic with notes on lead In: L.W. Chang, Editor, Toxicology of Metals, Lewis Publishers, Boca Raton, pp. 1027–1045.
Salvadori DM, Ribeiro LR, Oliveira MD, Pereira CA, Becak W (1992). The protective effect of beta-carotene on genotoxicity induced by cyclophosphamide. Mutat. Res., 265(2): 237-244.
Seenivasan S, Manikandan N, Muraleedharan N (2008). Chromium contamination in black tea and its transfer into tea brew. Food Chem., 106: 1066-1069.
Senaoana MP, Turner SD, van Apeldoorn GJ (1984). Research on rural non-farm employment in Lesotho: results of a baseline survey. Roma: Institute of Southern African Studies, National University of Lesotho: Research report 6.
Sharma RK, Agrawal M (2005). Biological effects of heavy metals: An overview. J. Environ. Biol., 26: S301-313.
Siegfried N, Parry CD, Morojele NK, Wason D (2001). Profile of drinking behaviour and comparison of self-report with the CAGE questionnaire and carbohydrate-deficient transferrin in a rural Lesotho community. Alcohol and Alcoholism, 36(3): 243-248.
Soylak M, Armagan F, Aydin A, Saracoglu S, Elci L, Dogan M (2002). Chemical Analysis of Drinking Water Samples from Yozgat, Turkey. Polish J. Environ. Studies, 11(2): 151-156.
Sunderman FW, Jr Kasprzak K, Horak E, Gitilitz P, Onkelinx C (1976). Effects of TETA upon the metabolism and toxicity of $NiCl_2$ in rats. Toxicol. Appl. Pharmacol., 38:177–188.
Templer DI, Griffin PR, Hintze J (1993). Gender life expectancy and alcohol: an international perspective. Int. J. Addict., 28(14): 1613-1620.
Turner S (2001). Livelihoods in Lesotho. CARE Lesotho, 5 April.
U.S. EPA . Environmental Protection Agency (1989). Guidance manual for assessing human health risks from chemically contaminated, fish and shellfish, U.S. Environmental Protection Agency, Washington, D.C. EPA-503/8-89-002.
U.S. EPA. Environmental Protection Agency (1995). Nickel. Integrated Risk Information System (IRIS), Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Cincinnati, OH.
Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem. Biol. Interact. 160: 1-40.
Varian AAS Handbook, Australia, 1988
VFAASAM. Varian Flame Atomic Absorption Spectrometry Analytical Methods (1989) Australia.
Wang X, Sato T, Xing B, Tao S (2005). Health risks of heavy metals to the general public in Tianjin, China via consumption of vegetables and fish. Sci. Total Environ., 350: 28-37.
WHO (2008). Who Guidelines for drinking-water quality [electronic resource]: Incorporating 1st and 2nd addenda, Vol.1, Recommendations. – 3rd ed.
WHO. World Health Organization (2011) Lesotho SOCIOECONOMIC CONTEXT.
WHO. World Health Organization (2011). WORLD HEALTH STATISTICS, 2011 http://www.who.int/whosis/whostat/EN_WHS2011_Full.pdf.
Wimer WW, Russell JA, Kappplan HL (1983). Alcohols Toxicology. Park Ridge New Jersey, Noyes Data Corporation.
Zacharski L, Chow B, Howes P, Lavori P, Shamayeva G (2004). Implementation of an iron reduction protocol in patients with peripheral vascular disease: VA cooperative study no. 410: The Iron (FE) and Atherosclerosis Study (FEAST)*1. American Heart J. 148(3): 386-392.
Zheng N, Wang QC, Zhang XW, Zheng DM, Zhang ZS, Zhang SQ (2007). Population health risk due to dietary intake of heavy metals in the industrial area of Huludao city, China. Sci. Total Environ., 387: 96-104.