Evaluation of Macro and Micro Elements in *Lawsonia inermis* (Mehndi)

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Summary: Thirty-five samples of different varieties of Henna (Mehndi) were analyzed for the detection of macro and micro elements and protein contents to assess their effect on hair. The concentration of micro elements, i.e., Zn, Mn, Cu, Co and Fe was found to be in the range of 0.002 to 0.033, 0.002 to 0.132, 0.001 to 0.01, 0.00012 – 0.0074 and 0.005 to 5.200 mg/g, respectively, whereas chromium was not detected in none of the samples analyzed. The concentration of the four macro elements studied, i.e., Ca, Mg, Na and K was found to be in the range of 0.219 – 39.273, 0.018 – 9.433, 0.40 – 10.0 and 0.095 – 17.0 mg/g respectively. Percentage of protein in branded and unbranded samples was found to be in the range of 9.824 - 13.024%, and in black Henna.25.989 to 27.114 %, which are comparatively higher then other samples.

Introduction

Henna is a naturally occurring plant (native to Middle East) is being used for the last thousand of years as a hair and body colorant. It is considered as a herb and has long been known to possess healing qualities. Henna dye is made from crushed dried leaves of henna plant (*Lawsonia inermis*), which contains an important cosmetic dye. The principle colouring mater is lawsone (*2-hydroxy-1,4-naptha-quinone*). The leaves are used as a stringent, acrid, refrigerant, vulnerary, anti-inflammatory, constipating as well as useful in wounds, ulcers, strangury cough, bronchitis, diarrhoea, dysentery, leprosy, boils, anaemia, opthalmia, fever, falling of hair and greyness of hair. It is also used as tropical sunscreen [1].

Henna, itself, does not come in a range of colours. The only dye molecule in henna (lawsone) in sufficient quantity, which stains hair and body, is a red-orange molecule. Any claim about achieving wide range of henna colours, with 100 % henna, using its shrubs, roots, barks or others parts has no truth. However, a wide range of henna colours may be achieved from synthetic dyes, made from a variety of ingredients and chemicals [2].

Black Henna

'Black henna' is the name given to an artificial product resulted by the addition of *para-phenylenediamine* (PPD) to natural henna, to create a black stain. PPD (a compound found to be carcinogenic in animal studies) is traditionally used in black hair dye, but it is a sensitizer, which means that it is something that the body is naturally allergic to or can become allergic to. Many hair dye makers prepare black henna by adding chemical compounds in henna, which may cause skin damage. Black henna can cause severe reactions that can take from 5-15 days to appear, which can cause permanent scarring and may even be life threatening. Reactions to black henna frequently necessitate immediate medical attention from a doctor or allergist. Most of the people suffer a skin reaction to permanent hair-colour. PPD is this colourless (hair-colour) precursor that under the influence of ingredients, such as ammonia and hydrogen peroxide, helps in the colour development in hair [3].

Allergies to Natural Henna

There are some people who are allergic to natural henna, but it is very rare. The reaction to pure henna is 'Type – I' allergic reaction. It is very unusual for person who is allergic to PPD in synthetic hair dyes is also being allergic to henna. This allergic reaction includes a tight feeling in chest, sneezing, itching eyes, running nose a day cough and hair loss [4, 5].

Allergies to Black Henna

Henna is often mixed with a para-phenylenediamine (PPD) mixture to force the

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resulting stain to black. The application of this mixture to the skin can cause rashes, immediately allergic reaction, and painful sores and will leave the victim permanently allergic. The danger of this chemical in henna use cannot be overstated. Henna combined with this chemical has been associated with angioneurotic edema, shock and renal failure in children and adults [6]. Sensitization to this chemical is a lifelong issue. It can mean problems with certain antibiotics and anaesthetics and should be dealt with immediately by a competent and knowledgeable physician and/or dermatologist.

PPD elicits not only contact hypersensitivity but immediate-type hypersensitivity. Not only that, but the sensitization issue seems to be much larger for another little known reason: subjects have been shown to react to lower concentrations of metabolic breakdown products of PPD than to the actual PPD molecule itself [7]. In the simplest terms, PPD breaks down in our body in a sort of digestive process.

Protein in Henna

Vitamins, minerals and protein maintain diet and reduce hair loss. Synthetic hair dyes penetrate into the cortex of hair and binding to natural pigment. In order to penetrate into the cortex, the cuticle of hair needs to be lifted that's why ammonia and hydrogen peroxide are used in synthetic hair dye. The repeated lifting of cuticle causes weathering of hair. Some cuticles are destroyed and some do not colour properly after repeated colouring. That's why coloured hairs loose lustre and shine over time. The ammonia/peroxide combination actually break down part, *i.e.,* keratin (protein) in hair each time when colour hair. Henna, on the other hand does not leave the cuticle, the way the regular hair colour neither does, or it breaks down the protein structure of hair. Intact, it strengthens hair as it combined with its own keratin to make hair stronger. That's why Henna acts as a natural protein treatment [1].

Now a days more and more people are colouring their hair; some use hair colour for added shine in hair, some for grey coverage, whereas others dye skin. In these studies we are concerned with the effects of chemicals and ingredients. These chemicals contain many essential, trace and toxic metals, and their lacking or presence in excess may affect hair texture, hair growth and skin. In addition to this, mineral balance is very important for hair growth and hair texture.

Vitamin and mineral supplement can also lead to mineral imbalance [8]. Calcium absorption is decreased in the presence of phosphorus [9]. Vitamin C is required for iron absorption but its presence in excess amounts may result in copper deficiency [9]. Vitamin D enhances Ca absorption, but its presence in excess may result in magnesium deficiency [9].

Trace minerals are essential to the countless metabolic functions in all phases of the life processes [9]. They not only provide the building blocks to life itself, but are also necessary in the production of hormones and enzyme activity. Minerals have interrelationship with every other mineral. Without optimum mineral level within the body, the other nutrients are not affectively utilized. The deficiency of a mineral or its presence in excessive quantity usually indicates its deficiency or excess within the body, or those minerals are bio unavailable. Dr Paul Eck, a leading proponent of hair analysis, called minerals as "sparkplug" of life, as these are involved in almost all enzyme reactions within our body [10]. Without enzyme activity, life is impossible to exist [11].

A trace mineral analysis is a preventive as well as a maintenance-screening tool. It allows us to keep a watchful eye on our own body's metabolism and to be able to take participate in the knowledge of inner working of our body. Improper nutritional intake, excessive ingestion of refined carbohydrates, sugars, a strict vegetation diet or other exclusive diets and use of hair dyes are common causes of mineral imbalances.

Continuous research shows that many health conditions can be contributed to various minerals imbalance. Bleaching and permanent hair dyes can cause an artificial elevation of calcium and magnesium. Zinc, iron, protein and other nutrients are responsible for hair growth. It has been recently observed that hair may be lost due to deficiency of Mg [12].

Copper is antagonist to zinc. Zinc deficiency has long been recognized as a factor in hair loses [13]. Iron and copper are the most common causes of hair loss. Chromium and manganese may be involved as causative agents in hair loss [13]. Our hair, scalp and skin have an electrical negative charge, whereas minerals and oxidizers are positively charged; when a positively charged mineral comes in contact with our hair, scalp and skin, it attaches on hair like a magnet.

That's why each and every mineral bears some effect on hair.

A number of 10 essential elements were analyzed and are categorized into macro and micro elements. Macro elements include Ca, Mg, Na and K that are needed in levels of over 100 mg/day and microelements (trace minerals) include Zn, Cu, Mn, Fe, Co and Cr that are needed in levels of under 100 mg/day [14]. These are present in body tissues in extremely small amounts but have critically important roles to play in human nutrition [15].

The branded samples are identified as "B", unbranded samples are identified as "U", Henna cone samples as 'V', arq-e-Henna samples as "A" and black Henna samples as "K"

This paper presents results of the study which was conducted to investigate the concentration of micro and macro elements and protein contents in different varieties of Henna (Mehndi) available in the various markets of Karachi city.

Results and Discussion

The mean value of six essential micro elements *i.e.* Zn, Cu, Mn, Fe, Co and Cr, estimated in different Henna samples are given in Tables-1a to 1e. Branded Henna samples in Table-1a, unbranded Henna samples in Table-Ib, Henna cone samples in Table-1c, arq-e-Henna samples in Table-1d and black Henna in Table-Ie. The mean value of four essential macro elements i.e. Ca, Mg, Na and K estimated in branded Henna samples are given in Table-2a, unbranded in Table-2b, arq-e-Henna in Table-2c, Henna cone samples in Table-2d and black Henna in Table-2e. Table-3 gives the percentage of protein content observed in branded, unbranded and black Henna samples. The essential values required for natural hair growth for micro and macro elements are given in Table-4 [16].

Table-1a: Concentration of Micro Elements (Zn, Mn, Cu, Cr, Co and Fe) recorded in Branded Henna.

Sample I.D	Zinc (mg/g)	Mn (mg/g)	Cu (mg/g)	Cr (mg/g)	Co (mg/g)	Fe (mg/g)
B1	0.022	0.103	0.0093	ND*	0.0019	2.75
B2	0.023	0.131	0.0100	ND*	0.0026	1.40
B3	0.031	0.120	0.0140	ND*	0.0014	5.20
B4	0.026	0.122	0.0120	ND*	0.0018	4.83
B5	0.033	0.118	0.0099	ND*	0.0027	5.30
B6	0.025	0.107	0.0110	ND*	0.0014	2.90
B7	0.025	0.114	0.0150	ND*	0.0039	2.45
B8	0.009	0.105	0.0095	ND*	0.0012	2.76
B9	0.028	0.128	0.0130	ND*	0.0020	3.15
B10	0.030	0.125	0.0120	ND*	0.0015	2.99
Mean	0.0252	0.1173	0.0116	--	0.0020	3.373
Std. Dev.	0.0067	0.0098	0.0019	--	0.0008	1.2954

* Not detectable in mg/g

Zinc

Zinc is a vital component of healthy hair and is responsible for cell production, tissue growth,

Table-1b: Concentration of Micro Elements (Zn, Mn, Cu, Cr, Co and Fe) recorded in Unbranded Henna.

Sample I.D	Zinc (mg/g)	Mn (mg/g)	Cu (mg/g)	Cr (mg/g)	Co (mg/g)	Fe (mg/g)
O1	0.028	0.099	0.013	ND*	0.0011	4.450
O2	0.024	0.086	0.011	ND*	0.0015	2.850
O3	0.022	0.108	0.017	ND*	0.0030	4.987
O4	0.020	0.104	0.010	ND*	0.0015	2.260
O5	0.024	0.101	0.011	ND*	0.0014	2.137
O6	0.027	0.132	0.013	ND*	0.0016	3.975
O7	0.029	0.090	0.014	ND*	0.0014	2.737
O8	0.020	0.050	0.012	ND*	0.0015	1.712
O9	0.026	0.090	0.012	ND*	0.0012	2.100
O10	0.024	0.118	0.015	ND*	0.0014	2.450
Mean	0.024	0.098	0.0128	--	0.0016	2.969
Std. Dev	0.003	0.0218	0.002	--	0.0005	1.1128

* Not detectable in mg/g

Table-1c: Concentration of Micro Elements (Zn, Mn, Cu, Cr, Co and Fe) recorded in Henna Cone.

Sample I.D	Zinc (mg/g)	Mn (mg/g)	Cu (mg/g)	Cr (mg/g)	Co (mg/g)	Fe (mg/g)
C1	0.009	0.03	0.004	ND*	0.00014	2.500
C2	0.007	0.015	0.003	ND*	0.00012	3.975
C3	0.004	0.017	0.001	ND*	0.00014	0.562
C4	0.004	0.014	0.004	ND*	0.00014	0.675
C5	0.002	0.018	0.002	ND*	0.00012	0.237
Mean	0.005	0.019	0.003	--	0.00013	1.590
Std. Dev	0.0027	0.0064	0.0013	--	1.095E-05	1.600

* Not detectable in mg/g

Table-1d: Concentration of Micro Elements (Zn, Mn, Cu, Cr, Co and Fe) recorded in Arq-e-Henna.

Sample I.D	Zinc (mg/g)	Mn (mg/g)	Cu (mg/g)	Cr (mg/g)	Co (mg/g)	Fe (mg/g)
A1	0.0030	0.002	0.002	ND*	0.0008	0.032
A2	0.0020	0.015	0.002	ND*	0.0004	0.121
A3	0.0030	0.113	0.001	ND*	0.0050	0.215
A4	0.0150	0.090	ND*	ND*	0.0074	0.115
A5	0.0020	0.121	ND*	ND*	0.0043	0.005
Mean	0.0050	0.068	0.001	--	0.0036	0.098
Std. Dev	0.0056	0.056	0.001	--	0.0033	0.083

* Not detectable in mg/g

Table-1e: Concentration of Micro Elements (Zn, Mn, Cu, Cr, Co and Fe) recorded in Black Henna.

Sample I.D	Zinc (mg/g)	Mn (mg/g)	Cu (mg/g)	Cr (mg/g)	Co (mg/g)	Fe (mg/g)
K1	ND*	0.006	0.007	ND*	0.0012	0.102
K2	ND*	0.008	0.005	ND*	0.001	0.243
K3	ND*	0.005	0.005	ND*	0.0014	0.110
K4	ND*	0.002	0.002	ND*	0.001	0.105
K5	ND*	0.005	0.004	ND*	0.0012	0.216
Mean	--	0.005	0.005	--	0.0012	0.155
Std. Dev	--	0.002	0.0018	--	0.0002	0.0685

* Not detectable in mg/g

Table-2a: Concentration of Macro Elements (Ca, Mg, Na and K) recorded in Branded Henna.

Sample I.D	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Na/K ratio
B1	19.565	5.880	3.0	8.0	0.375
B2	35.156	3.125	2.5	7.5	0.333
B3	31.904	6.904	5.0	16.0	0.313
B4	18.287	3.240	5.0	8.5	0.588
B5	39.273	9.433	5.0	14.0	0.357
B6	22.994	4.261	7.5	14.5	0.517
B7	35.817	6.490	6.0	13.5	0.444
B8	25.555	5.567	7.5	12.5	0.600
B9	20.985	4.231	7.0	9.0	0.777
B10	31.657	7.006	3.0	9.5	0.315
Mean	28.119	5.614	5.15	11.3	4.619
Std. Dev.	7.554	1.961	1.872	3.120	0.1552

Table-2b: Concentration of Macro Elements (Ca, Mg, Na and K) recorded in Unbranded Henna.

Sample I.D	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Na/K ratio
O1	28.883	5.097	10.0	15.0	0.666
O2	28.452	7.142	5.0	13.5	0.370
O3	32.943	6.775	10.0	14.5	0.689
O4	32.001	5.092	7.5	13.5	0.555
O5	32.059	5.089	5.0	14.0	0.357
O6	23.084	6.250	10.0	17.0	0.588
O7	23.725	3.941	7.5	7.0	1.071
O8	25.295	3.816	5.0	9.0	0.555
O9	35.891	5.198	5.0	15.0	0.333
O10	27.884	6.154	7.5	5.5	1.363
Mean	29.022	5.455	7.25	12.4	0.656
Std. Dev.	4.210	1.113	2.189	3.836	0.329

Table-2c: Concentration of Macro Elements (Ca, Mg, Na and K) recorded in Arq-e- Henna.

Sample I.D	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Na/K ratio
A1	0.414	0.071	0.55	0.20	2.75
A2	0.555	0.095	0.60	0.20	3.00
A3	0.369	0.141	0.45	0.50	0.90
A4	0.347	0.058	0.55	0.30	1.83
A5	0.219	0.534	0.40	0.25	1.60
Mean	0.381	0.180	0.51	0.29	2.016
Std. Dev.	0.121	0.201	0.082	0.125	0.860

Table-2d: Concentration of Macro Elements (Ca, Mg, Na and K) recorded in Henna Cone.

Sample I.D	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Na/K ratio
C1	15.09	2.028	2.50	7.50	0.333
C2	13.942	1.880	3.75	4.50	0.833
C3	11.556	1.415	1.25	3.75	0.333
C4	16.666	2.101	1.45	12.75	0.114
C5	18.500	2.314	1.25	2.50	0.500
Mean	15.151	1.948	2.04	6.20	0.423
Std. Dev	2.641	0.336	1.088	4.098	0.155

Table-2e: Concentration of Macro Elements (Ca, Mg, Na and K) recorded in Black Henna.

Sample I.D	Ca (mg/g)	Mg (mg/g)	Na (mg/g)	K (mg/g)	Na/K ratio
K1	1.315	0.111	8.0	0.75	10.66
K2	0.799	0.018	7.5	1.25	6.00
K3	1.026	0.055	7.5	0.80	9.375
K4	0.856	0.036	8.5	0.50	17.00
K5	1.051	0.511	7.5	0.95	7.895
Mean	1.009	0.146	7.8	0.68	10.186
Std. Dev.	0.202	0.207	0.447	0.424	1.860

Table-3: Percentage of Protein observed in of Branded, Unbranded and Black Henna.

Branded Henna Sample ID	Percentage of Protein (%)	Unbranded Henna Sample ID	Percentage of Protein (%)	Black Henna Sample ID	Percentage of Protein (%)
B1	11.182	O1	10.996	K1	27.114
B2	12.263	O2	10.965		
B3	12.114	O3	9.824	K2	26.631
B4	11.208	O4	10.124		
B5	9.625	O5	11.014	K3	26.872
B6	13.024	O6	10.317		
B7	11.529	O7	10.448	K4	25.989
B8	12.191	O8	9.897		
B9	12.212	O9	10.226	K5	26.102
B10	12.008	O10	10.811		
Mean	11.736	Mean	10.462	Mean	26.542
Std. Dev.	0.923	Std. Dev.	0.457	Std. Dev.	0.4343

Table-4: Essential Values of Elements Required for Natural Hair Growth.

Microelements	Lower limit (mg/g)	Higher limit (mg/g)
Zinc	0.090	0.265
Copper	0.007	0.040
Manganese	0.0002	0.004
Iron	0.020	0.200
Cobalt	0.00005	0.0007
Chromium	0.0002	0.003
	Macroelements	
Calcium	0.200	1.00
Magnesium	0.028	0.10
Sodium	0.050	0.35
Potassium	0.030	0.20

repair and maintenance of oil secreting glands of scalp. It also plays a large role in protein synthesis and collagen formation; that is why zinc is important for both hair maintenance and dandruff prevention. A lack of this mineral can also slow down the growth of hair [17, 18]. From Tables (1a-1d) it can be seen that the analyzed branded, unbranded Henna, Henna Cone and Arq-e-Henna samples contain 0.0090 to 0.033, 0.020 to 0.029, 0.002 to 0.009 and 0.002 to 0.015 mg/g zinc, respectively, whereas Zn was not detected in black Henna.

Copper

This trace mineral is necessary in the production of haemoglobin and haemoglobin is vital for the process of carrying oxygen to the tissues and to the hair follicles. Its excess can discolour hair causing blonde hair to turn green and dark hair to tint darker. Too much copper can inhibit both zinc and iron absorption. Like Magnesium; it can inhibit the proper processing of perm, colour, and relaxers. It weighs down hair and causes dryness [17, 18]. The results show that the analyzed branded, unbranded, Henna cone, arq-e-Henna and black Henna samples contain 0.0093 to 0.015, 0.010 to 0.017, 0.001 to 0.004, 0.001 to 0.002 and 0.002 to 0.007 mg/g copper, respectively.

Manganese

The Lack of this mineral can slow down hair growth and change hair colour. High hair manganese level indicates problem with calcium and iron metabolism [19]. The analyzed samples of branded, unbranded, Henna cone, arq-e-Henna and black Henna contain 0.103 to 0.131, 0.050 to 0.132, 0.014 to 0.030, 0.002 to 0.121 and 0.002 to 0.008 mg/g manganese respectively.

Iron

Iron is also essential for hair, but its presence in excess can cause dark hair to tint darker and blonde hair to turn orange. It can block perms and colour for properly processing. Iron can dry, brittle and weighted down hair [17, 18]. Like Cu, it is also essential for synthesis of haemoglobin, which carries oxygen to hair follicle. The results show that the analyzed samples of branded, unbranded, Henna Cone, Arq-e-Henna and Black Henna contain 1.40 to 5.30, 1.712 to 4.987, 0.237 to 3.975, 0.005 to 0.215 and 0.102 to 0.243 mg/g iron respectively.

Cobalt

Cobalt pigments produce light brown shades of hair [20]. The concentration of cobalt in the branded, unbranded, Henna Cone, Arq-e-Henna and Black Henna was recorded in the range of 0.0012-0.0039, 0.0011-0.0030, 0.00012-0.00014, 0.00040-0.0074 and 0.001-0.0014 mg/g respectively.

Chromium

Chromium is also not found in all samples of Henna. Chromium is an essential trace element required for the maintenance of normal blood sugar and cholesterol levels. Diffuse hair loss, diabetes-like symptoms, and fatigue may indicate a lack of chromium. People whose dietary intake is high in processed foods will commonly be deficient in chromium. Other problems that can affect chromium status are a low vitamin B6, or iron overload [21].

Calcium

Calcium is a vital macro mineral, necessary for hair growth, but its presence in excess may cause many problems, such as hair feeling dry and weighted down. It builds up on the scalp, causing flaking of scalp, giving the appearance of dandruff at the mouth of follicle causing hair to break off, and then coating the scalp blocking further new hair growth, whereas its deficiency brittles the hair and nail [22, 23]. In this study, it is observed that in the analyzed samples of branded, unbranded, Henna Cone, arq-e-Henna and black Henna, calcium contents were observed in the range of 18.287-39.273, 23.084-35.891, 11.556-18.500, 0.219-0.555 and 0.799-1.315 mg/g respectively.

Magnesium

Like calcium, magnesium is also an essential macro mineral for the hair growth. Excess magnesium causes hair to lack shine. It can inhibit the proper processing of perms, colour and relaxers and causes hair thin and dry [22, 23]. The conc. of magnesium in branded, unbranded, Henna Cone, arq-e-Henna and black Henna samples was observed in the range of 3.125 to 9.433, 3.816 to 7.142, 1.415 to 2.314, 0.058 to 0.534 and 0.018 to 0.511 mg/g respectively.

Sodium and Potassium

Sodium-Potassium ratio is an important factor for hair growth and for healthy nervous system. An unbalanced Na/K ratio is associated with a compromised immune system and lowered energy level. It is also associated with adrenal weakness and tendency for carbohydrate intolerance [17, 18]. Dr. Eck found the ideal sodium/potassium ratio is about 2.5:1 in an unwashed sample of head hair [24]. The established normal ratio for sodium and potassium (Na:K), relative to the adrenal glands, was 2.5:1. A perfect Na:K ratio (*i.e.* 2.5:1) suggests optimum adrenal gland function, attempting to maintain good health. It is a very comprehensive form of mineral feedback that reveals to us how we have been affected by various stressors in our life [24]. The conc. of sodium in the branded, unbranded, Cone, arq-e-Henna and black Henna samples was recorded in the range of 2.50 to 7.5, 5.00 to 10.00, 1.25 to 3.75, 0.40 to 0.60 and 7.5 to 8.5 mg/g, respectively and potassium in the range of 7.50 to 16.00, 5.50 to 17.00, 2.50 to 12.75, 0.20 to 0.50 and 0.50 to 1.25 mg/g, respectively.

The results show that as compared to the essential values required for hair growth [16] given in Table-4, the concentration of (i) zinc in all the samples of Henna was found below the lower values recommended for hair growth, (ii) manganese, copper and cobalt was found within the

recommended limits and (iii) iron exceeds the upper limit and it leaves side effect on hair [17, 18]. Chromium was not detectable an all the samples of Henna. The conc. of calcium, magnesium, sodium and potassium was also recorded above the upper limit recommended for hair growth. Excess Calcium and magnesium cause many hair problems [22, 23]. An unbalance Na/K ration and presence of Na and K above the recommended limit may also result in adverse effect on hair growth [24]

Experimental

Sampling

In this study, a total number of 35 samples of different varieties of Henna (including 10 branded samples, 10 unbranded samples, 05 samples of cone, 05 samples of Black Henna (Kali Mehndi) and 05 samples of Arq-e-Henna, were randomly collected from the markets in different areas and localities of Karachi.

Analytical Procedure

All powdered samples were first dried at 105°C, then one gram of each variety of sample was taken in an acid washed beaker and were chemically analyzed for the detection of four macro (Ca, Mg, Na and K) and six micro (Zn, Cu, Mn, Fe, Co and Cr) elements, using wet digestion technique by Nitric Acid and Hydrogen Peroxide [25]. Analyses were generally carried out using Atomic Absorption Spectrophotometer (Hitachi Model Z-5000), equipped with Zeeman background corrector and data processor, ZAA. Sodium and Potassium were analyzed by Flame Photometer (Model Corning 410). Analytical reagent grade chemicals were used in the preparation of samples and standards. Mean values of duplicate samples were considered. Wet samples, *i.e.* Cone and Arq were digested without drying. Protein content was analyzed by Kjeldhal Nitrogen Method [25].

Reagents and Glassware

Analytical reagent grade chemicals and de-ionised water were used in the preparation of samples and standards. Caliberation standards were made by dilution of high purity grade Merck metal standards. All the glassware used in the analysis were soaked in 1 %ˆNitric acid for one day then washed several times with tap water and finally washed with plenty of dë-ionised water and dried in an oven at 100 °C.

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