Comparison of metal ions release and corrosion potential from different bracket archwire combinations
(An in vitro study)

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ABSTARCT

Background: Esthetic treatment is the options of patient seeking orthodontic treatment. Therefore this study was conducted to measure the concentration of Aluminum, Nickel, Chromium and Iron ions released from combination of monocrysralline brackets with different arch wires immersed in artificial saliva at different duration, to evaluate the corrosion point on different parts of the orthodontic appliances before and after immersion in artificial saliva, and to evaluate the corrosion potential of each group of the orthodontic appliances.

Material and methods: Eighty orthodontic sets prepared. Each set represents half fixed orthodontic appliance, from the central incisor to the first molar, for the maxillary arch, each set consisted of molar band, five brackets, half arch wire and ligature elastic.These sets are divided into two groups: **Group A**: with monocrystalline brackets divided into five subgroups (each subgroup has ten sets), but differ in arch wires, as numbered stainless steel, nickel-titanium, thermally activated, coated stainless steel and coated nickel-titanium arch wires respectively. **Group B**: with stainless steel brackets divided into three subgroups (also each subgroup has ten sets), but differ in arch wires, as numberedstainless steel, nickel-titanium, and thermally activated arch wires respectively. Used optical microscope to check the corrosion points, and used potentiostat techniques to indicate corrosion rate and tendency.

Results: The greatest concentration of Aluminum and nickel ions release during the 1st week in group **A**, then sharply decreased in the 2nd week. The release of chromium ion released increase with increase intervals, while iron ion released decrease with increase time. Both nickel and chromium ions increase with increase intervals in group **B**,while iron increase to the maximum at 3rd weeks, then began to degrease. Optical microscope displayed pitting, crevices, and intergranular corrosion. Potentiostat techniques indicated that increase corrosion when used stainless steel and coated nickel titanium than others arch wires with group **A**, while corrosion increase with nickel titanium than stainless steel arch wires with group **B**.

Conclusions: Non-significant difference in the total nickel, chromium and iron release in group B. Aluminum and iron increase in A4 and A5, while nickel and chromium increase in A1 and A2.The total released amounts of metals ions in both groups were less than the amounts of daily intake.

Key words: ions release, aluminum, nickel, chromium, iron. **(J Bagh Coll Dentistry 2014; 26(1):171-179).**

INTRODUCTION

In orthodontics a broad spectrum of stainless steels (1,2) and Ti-based alloys (3)are used for the production of brackets and arch wires although the latter are mainly fabricated from Ni-Ti alloys (4).

Ceramic brackets when introduced overcome the partial of problems with aesthetic demand. Ceramic brackets largely overcome the esthetic limitations of plastic brackets in that they are quite durable and resist staining. In addition, they can be custom-molded for individual teeth and are dimensionally stable, so that the precise bracket angulations and slots of the straight-wire appliance can be incorporated. Most currently-available ceramic brackets are produced from alumina, either as single-crystal (sapphire) or polycrystalline (ceramic) units came into the field of orthodontics. Single-crystal brackets should offer greater strength than polycrystalline brackets, and fracture resistance is reduced to or below the level of the polycrystalline materials.

Because of the multiple crystals, polycrystalline alumina brackets have relatively rough surfaces, even though monocrystalline alumina is as smooth as steel, these brackets also show greater friction than steel, perhaps reflecting a chemical interaction between the wire and bracket material (5).

A number of alternatives have been explored to create an esthetic arch wire that would allow efficient orthodontic treatment from the labial aspect (6).Materials used in coating are polymers such as synthetic fluorine-containing resin or epoxy resin composed mainly of polytetrafluoroethylyene, which is used to simulate tooth color (7). These metallic arch wires coated with colored polymers or inorganic materials are currently the solution to this esthetic problem (8).

The purpose of this in vitro study is to determine the ions release, corrosion points and corrosion tendency when used monocrystalline brackets with different arch wires and also compare them with stainless steel arch wires.

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MATERIALS AND METHODS

Eighty orthodontic sets prepared. Each set represents half fixed orthodontic appliance, from the central incisor to the first molar, for the maxillary arch, these preparation and measurement were taken from model which prepared with fixed orthodontic appliance.Each set consisted of molar band of similar size and closely weight, five brackets, half arch wire and finally ligated by ligature elastic.These sets are divided into two groups, **Group A**: divided into five subgroups (each subgroup has ten sets, all with monocrystalline brackets), these subgroups numbered according to the arch wires: A1 stainless steel arch wires, A2 nickel-titanium arch wire, A3 thermally nickel-titanium arch wire, A4 coated stainless steel arch wire, and A5 coated nickel-titanium arch wire. **Group B**: divided into three subgroups (also each subgroup has ten sets, all with stainless steel brackets),these subgroups numbered according to the arch wires: B1 stainless steel arch wires: B1 stainless steel arch wires, B2nickel-titanium arch wire, and B3thermally nickel-titanium arch wire. The samples immersed in ethanol for 4-5 seconds and then immersed in acetone for 8 seconds and dried in hot air and restored again, this is to get rid of any contaminants during ligation.

Neither the inner surface of the bands, nor the mesh of the brackets were covered by any material so that the exposed surface area of the appliance components was approximately equal to the exposed surface area of the bonded and banded full arch fixed orthodontic appliance (9).

The artificial saliva which is testing solutions (The electrolytic media) used in this study consisted of 0.7g NaCl, 1.2g KCl, 0.26g Na_2HPO_4, 0.2g K_2HPO_4, 1.5g $NaHCO_3$, 0.33g KSCN, 0.13g urea and 1000 ml deionized water, PH of the saliva adjusted to 6.7 ± 0.10 using the pH- meter, this pH value coincides with that reported of human saliva (9).

Each sample was placed in glass container, filled with 30 ml of artificial saliva, and closed by parafilm to control evaporation. To avoid saturation with the released ions during the incubation period, the solutions were changed four times with 7 days intervals (10). To measure ions [aluminum (Al), nickel (Ni), and chromium (Cr)]used flameless Atomic absorption spectrophotometer (AAS) concentration (Buck scientific/graphite furnace, 210 VGP, USA), while used spectrophotometer (Cecil, 1011, France) to measure iron (Fe) concentration.Microscopical examination was done using optical microscope (MTI corporation, 2700, camera 94804, USA), performed to determine the position and severity of corrosion in the components of fixed orthodontic appliance with 100X magnification. The Potentiostat (M Lab 200, Germany) used to determine the E-corr (corrosion tendency) and I-corr (corrosion rate) with different appliances combinations.

RESULTS

The greatest concentration of Al ions release during the 1st week in all subgroup of group A, then sharply decreased in the 2nd week with significant difference between 1st week with all others weeks.The greatest Ni release for A1, A4 and A5 noticed in the 1st week, while A2 and A3 in the 4th week then reduced at day (14), increased in (21) days, and the release then followed a regular pattern at the final intervals for all types of the appliances combination.The release of Cr increase with intervals until reach the greatest level in the 3rd week for A1, A2, A3 and A4, and then decrease in 4th intervals,while in group A5 the Cr increases with time to reach maximum at 4th week.The greatest Fe ion released in the 2nd week in A1 and A3, while in the 1st week in A2, A4 and A5 Tables (1-5).In group Bthe amount of Ni and Cr ions increased with increased intervals for all subgroups.Fe ions increased as the immersion time progressedespecially at 3rd weeks, and then decreased at 4th weeks Table (6-8).

The wing of the monocrystalline bracket was subjected to pitting and crevice corrosion appeared in all groups, but large pitting apparent in A1, while crevice more apparent in A2 and A3 Figure 1, and no clearly appearance of corrosion in slots.The wings of stainless steel brackets showed slightly defect especially in B1 and B2 Figure 2,while slot showed only minor pitting corrosion occur.The welder area between band and tube reflect intergranular corrosion, reveled more in groups with NiTi arch wires Figure 7.Stainless steel orthodontic arch wiresidentified clearly pitting and crevice corrosion in both bracket and band position Figure 3.NiTi arch wires showedclearly pitting and with crevice corrosion in bracket position while more clearly appearance of large pitting corrosion in B2 Figure 4.Thermally activated NiTi arch wires showed clearly pitting and slightly crevice corrosion in bracket position while more clearly exhibition of large pitting corrosion in both A3 and B3 at band position Figure 5.New coated stainless steel arch wiresdisplayed slightly solving the coated materials, with pitting corrosion in the uncoated area. In contact area also displayed solving the coated material with slightly pitting corrosion.

New coated NiTi arch wires displayed clearly pitting corrosion at bracket position after tear out coated area during used period. While band position were tested after used indicated more pitting and crevice corrosion on uncoated area. In contact area between coated and uncoated displayed pitting corrosion Figure 6.

E-corr and I-core values for the group A according to groups was as following Table (9),E-corr and I-core values for the group B according to groups was as following Table (10).

DISCUSSION

Although aluminum oxide or alumina (Al_2O_3) is an extremely stable compound with a high melting point, however consider all possible reactions. Suppose the first step of the reaction is the reduction of Al^{3+} and oxidation of the alkali metal (M)

$Al_2O_3 + tM \dashrightarrow uM_vO_w + xAl_yO_z$…. (1)

The reaction with the most favorable change in energy for all alkali metals is:

$Al_2O_3 + 6M \dashrightarrow 3M_2O + 2Al$ …… (2)

These reactions allow Al to be released; these reactions contribute a lot of energy to the overall reaction. For this reason oxygen has a detrimental effect on the alkali metal resistance of alumina (11).

The reduction in the Al ions concentration between the first and the other weeks may be due to the (3) reaction, that after released Al ions then react with water to the reformation of the Al_2O_3 as following:

$2Al + H_2O \rightarrow Al_2O_3 + 3H_2$ ………. (3)

The results also may be due to (4) reaction, according to the research suggested that the enhancing effect of aluminum oxide on the reactivity of aluminum with water may also be mechanochemical in nature (12).

$Al_2O_3 + 3H_2O \rightarrow 2AlOOH$ ……… (4)

$AlOOH$ film growth and formation of H_2 bubbles

$6AlOOH + 2Al \rightarrow 4Al_2O_3 + 3H_2$

After reduction in of Al in second week, there were slightly increase with intervals in A1 and A5 with non-significant differences between 2nd week with 3rd week, and 3rd week with 4th week, and these mainly related to the stability of the alumina formation by the reactions (3) or (4), also may be related to the arch wires. While in A2, A3, and A4 after reduction in of Al in 2nd week, still continuous reduction with intervals, this related to the stability of the alumina formation by the reactions (3) or (4) which was more stable in these groups than previous groups, also may be related to the arch wires.

The greatest Ni release for A1, A4 and A5 was in the 1st week, therefore stainless steel and both coated arch wires taken the same pattern of corrosion with monocrystalline brackets, while A2 and A3 in the 4th week, from that NiTi and thermally NiTi took the same pattern of corrosion with these brackets.The release of Cr increase with intervals until reach the greatest level in the 3rd week for A1, A2, A3, and A4, and then decrease in four intervals, these indicated that stability of the formation of the passive film began after 3rd week. While in A5 the Cr increase with increase time to reach maximum at 4th week, this indicated that in the presence of the coated NiTi arch wire will delay formation of passive film by reduction of the oxygen.The greatest Fe ion released in the 2nd week for A1 and A3, while in the 1st week for A2, A4 and A5, the release then reduced with increase time, because of the low amount of chromium comparing with the high amount of iron, it may not enough to produce such strongly adherent passivation layer of chromium oxide on the surface to prevent corrosion of iron (13). There was non-significant difference of total amount of Al ion between A1, A2, and A3 even with different arch wires; this indicated that monocrystalline brackets took similar degree of corrosion with the uncoated arch wires, also there was non-significant difference between A4 and A5 and this may be indicated that monocrystalline bracket took similar degree of corrosion with coated arch wires.

The greater total amount of Ni and Cr ions release in A1 and this may be due to the reaction (1) or (2), the composition percentage, and/or passive film stability. The chromium oxide passive films are not as stable as their titanium oxide and thus contribute to the inferior corrosion resistance of stainless steel relative to Ti alloys (14,15), therefore more (Ni and Cr) ions released in A1 than other groups.

Total amount of Fe ion release, more in coated than uncoated arch wires groups, these results depend specially on the manufacturing of the wires, and according to the researches showed that coated arch wires had low esthetic value after clinical used. The remaining coating showed a severe deterioration and a greater surface roughness compared to preclinical used (16). For the Ni and Cr ions, the greatest amount with B1, B2 and B3 noticed in the 4th week. The amount of Ni and Cr ions increased with intervals for all types of the appliances combination. These agreement with studies (17-19). But disagree with others (9,20-22). The results showed that the release of Fe ions increased especially at 3rd weeks, and then decreased at 4th weeksthese agreement withstudies (15,18,23) and disagree with others (22).At the end of 28 days, the total Ni, Cr and Fe release were non-significant difference in the levels in different groups, this agree with study (24).

The total amount of Ni, Cr and Fe ions in all subgroups of both main groups were less than the cumulative daily intake. Quantities of all released ions were below the toxic levels and did not exceed daily (9,18,24,25).

Corrosion in the form of pitting has been identified in brackets and wires, initiation of the process may take place before immersion placement since excessively porous surfaces have been found on as-received, these agrees with other studies (26,27). Also crevice corrosion is obvious at different parts including bracket slots, the arch wire side at the bracket slot and the part passes through molar band tube. These agree with those mentioned by others (26). The crevice sites represent a harbor of stagnant solution and under the deposit area, oxygen depletion takes place, no further oxygen reduction occur, although the dissolution of the metal continues, this tend to produce an excess of positive charges in the solution that is balanced by migration of chloride ions to the crevice, caused increase concentration of metal-chloride within the crevice (28).

Microscopically examined of the arch wires both stainless steel and NiTi showed evidence of pitting formed on the wire surfaces, these agreement with other study (29). However, surface irregularities observed in NiTi arch wires, which are sites susceptible to selective dissolution of Ni may arise from the manufacturing process, since the presence of manufacturing defects may accelerate the corrosion process (14,30).

NiTi and Thermally NiTi arch wires showed pitting corrosion more with stainless steel than monocrystalline groups due to a galvanic reaction. Coated NiTi showed more corrosion than coated stainless steel arch wires. The surface defects produced during the manufacturing processes in molar band near the welded area, such manufacturing defect was observed by other (31), reveled more corrosion in groups with NiTi arch wires due to a galvanic reaction will occur.

Potentiostatic Polarization Curves associated with group A depend on the arch wire and band because monocryatalline brackets were insulator for electrical current. A1 and A2, both (E-corr) and (I-corr) with stainless steel indicated more corrosion tendency and higher corrosion rate than with NiTi arch wires. These results were coincidence with the results of Atomic absorption Spectrophotometer and Spectrophotometer. While A3, both (E-corr) and (I-corr) indicated less corrosion and higher corrosion rate than above. Both (E-corr) and (I-corr) with A5 indicated more corrosion tendency and higher corrosion rate with coated NiTi than coated stainless steel arch wires with A4, also microscopical examination indicated more corrosion with coated NiTi arch wires. In groups of stainless steel brackets, Potentiostatic Polarization Curves associated with arch wire, band and brackets. B1 and B2, the analysis of the curves reveals the following:

1. The corrosion potential (E-corr) was higher in stainless steel than nickel-titanium arch wire.
2. The values of (I-corr) were higher in nickel-titanium than in stainless steel arch wire.
3. In B3, E-corr indicated less corrosion tendency, but high I-corr indicated higher corrosion rate.

When contact with a dissimilar metal is made, the self-corrosion rates will change, corrosion of the anode will accelerate; corrosion of the cathode will decelerate or even stop. The two major factors affecting the severity of galvanic corrosion are (1) the voltage difference between the two metals, and (2) the size of the exposed area of cathodic metal relative to that of the anodic metal. Corrosion of the anodic metal is both more rapid and more damaging as the voltage difference increases and as the cathode area increases relative to the anode area.

E-corr values for appliances contain stainless steel arch wire was agreed with other studies (9,26). I-corr values it was higher in the presence of NiTi and thermo NiTi as compared with the presence of stainless steel arch wires, also agree with other study (9).

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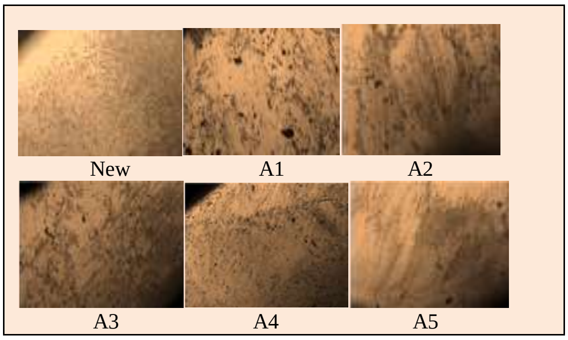

Figure 1: Monocrystalline bracket

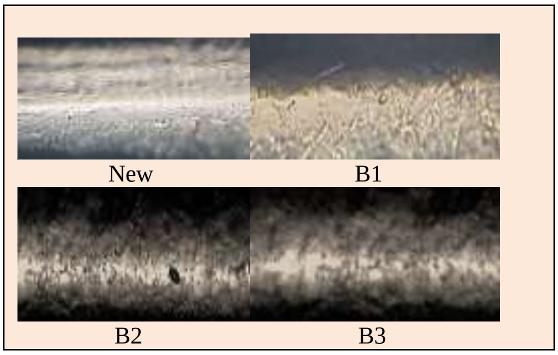

Figure 2: Stainless steel bracket

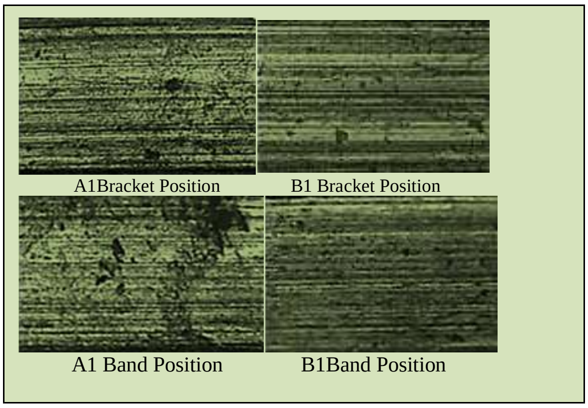

Figure 3: Stainless steel arch wire

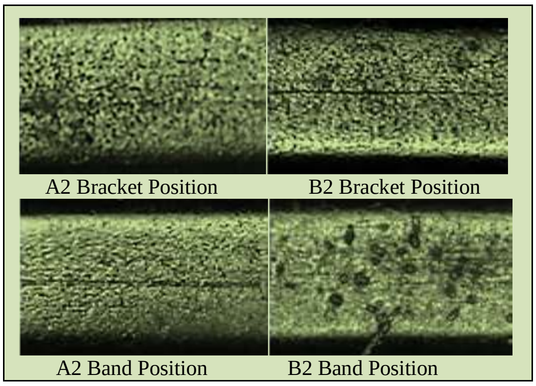
A2 Bracket Position B2 Bracket Position
A2 Band Position B2 Band Position

Figure 4: Nickel titanium arch wire

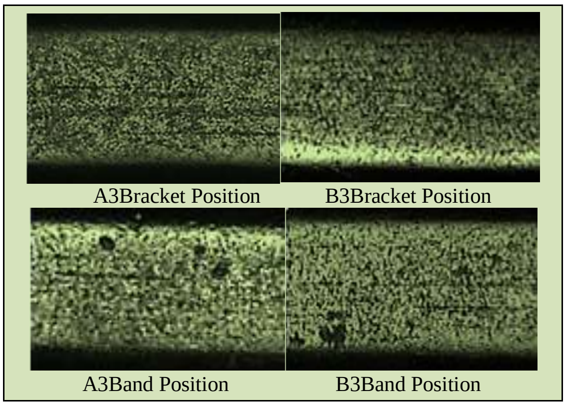
A3Bracket Position B3Bracket Position
A3Band Position B3Band Position

Figure 5: Thermally Nickel titanium arch wire

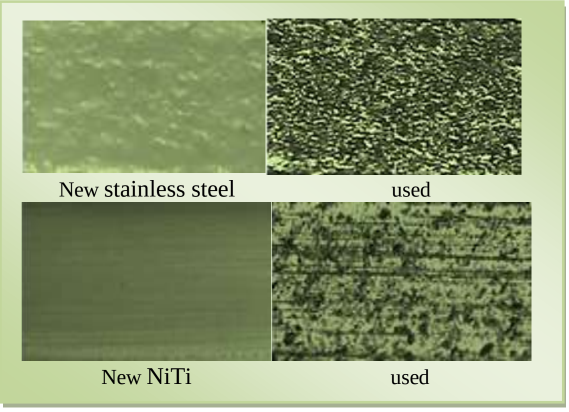
New stainless steel used
New NiTi used

Figure 6: Coated arch wire

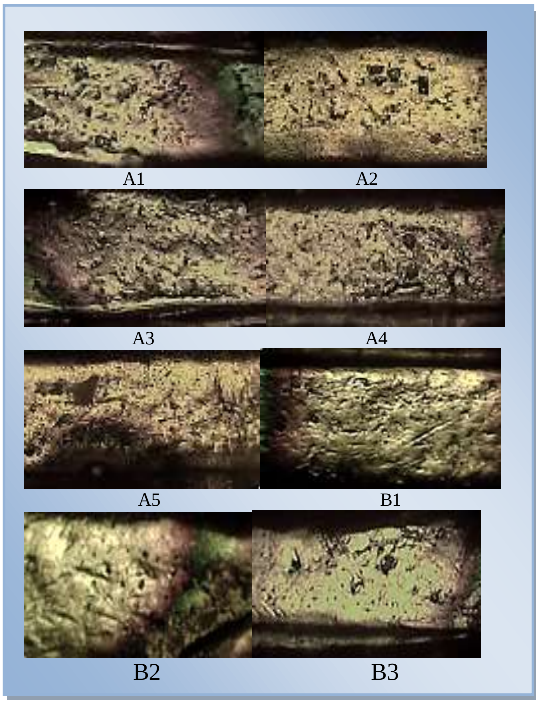

Figure 7: Band

Table 1: Concentration (μg/ml) A1

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Al	**1st week**	0.00529	0.00247	0.00078	0.0023	0.0091	16.38	0.000 (HS)
	2nd week	0.001689	0.00021	0.00007	0.00129	0.00192		
	3rd week	0.002154	0.00012	0.00004	0.00199	0.00233		
	4th week	0.00313	0.00030	0.00010	0.00256	0.00341		
Ni	**1st week**	0.01396	0.00261	0.00082	0.0089	0.0182	151.475	0.000 (HS)
	2nd week	0.002169	0.00048	0.00015	0.00125	0.00281		
	3rd week	0.003908	0.00011	0.00003	0.00371	0.00402		
	4th week	0.007264	0.00036	0.00011	0.00681	0.00774		
Cr	**1st week**	0.000352	0.00010	0.00003	0.00019	0.00051	32.593	0.000 (HS)
	2nd week	0.000521	0.00013	0.00004	0.00029	0.0007		
	3rd week	0.000698	0.00002	0.00001	0.00066	0.00073		
	4th week	0.000446	0.00002	0.00001	0.00042	0.00047		
Fe	**1st week**	0.3202	0.033419	0.010568	0.288	0.392	13.156	0.000 (HS)
	2nd week	0.3608	0.083518	0.026411	0.248	0.472		
	3rd week	0.3264	0.047673	0.015076	0.248	0.384		
	4th week	0.2208	0.025633	0.008106	0.184	0.256		

Table 2: Concentration (µg/ml) A2

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Al	1st week	0.00468	0.00175	0.00055	0.0025	0.0081	15.907	0.000 (HS)
	2nd week	0.002628	0.00023	0.00007	0.00208	0.00287		
	3rd week	0.002696	0.00010	0.00003	0.00252	0.00283		
	4th week	0.002166	0.00011	0.00004	0.00201	0.00232		
Ni	1st week	0.00552	0.00212	0.00067	0.0023	0.0098	23.578	0.000 (HS)
	2nd week	0.002717	0.00048	0.00015	0.00226	0.00357		
	3rd week	0.003561	0.00004	0.00001	0.00349	0.00361		
	4th week	0.006339	0.00024	0.00008	0.00598	0.00665		
Cr	1st week	0.000195	0.00004	0.00001	0.00012	0.00025	29.382	0.000 (HS)
	2nd week	0.000348	0.00016	0.00005	0.00019	0.00071		
	3rd week	0.000564	0.00007	0.00002	0.00048	0.00069		
	4th week	0.000408	0.00001	0.00000	0.0004	0.00042		
Fe	1st week	0.3152	0.02269	0.007175	0.264	0.336	38.564	0.000 (HS)
	2nd week	0.3072	0.044173	0.013969	0.248	0.384		
	3rd week	0.1866	0.038598	0.012206	0.136	0.242		
	4th week	0.2128	0.02173	0.006872	0.184	0.248		

Table 3: Concentration (µg/ml) A3

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Al	1st week	0.00634	0.001862	0.000589	0.0028	0.0093	46.116	0.000 (HS)
	2nd week	0.003462	0.00023	0.00007	0.00315	0.00382		
	3rd week	0.00269	0.00004	0.00001	0.00263	0.00275		
	4th week	0.00154	0.00033	0.00011	0.00121	0.00202		
Ni	1st week	0.00403	0.00120	0.00038	0.0023	0.0059	94.520	0.000 (HS)
	2nd week	0.001107	0.00015	0.00005	0.00089	0.0013		
	3rd week	0.003272	0.00010	0.00003	0.00312	0.00345		
	4th week	0.005588	0.00009	0.00003	0.00544	0.00571		
Cr	1st week	0.000534	0.00013	0.00004	0.00034	0.00076	16.095	0.000 (HS)
	2nd week	0.000307	0.00008	0.00003	0.00019	0.00044		
	3rd week	0.000502	0.00005	0.00002	0.00044	0.00061		
	4th week	0.000391	0.00001	0.00000	0.00038	0.0004		
Fe	1st week	0.344	0.069128	0.02186	0.248	0.432	43.998	0.000 (HS)
	2nd week	0.4496	0.063978	0.020232	0.352	0.536		
	3rd week	0.2418	0.0406	0.012839	0.174	0.292		
	4th week	0.2	0.028472	0.009004	0.176	0.256		

Table 4: Concentration (µg/ml) A4

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Al	1st week	0.00871	0.00079	0.00025	0.0076	0.0099	499.376	0.000 (HS)
	2nd week	0.003462	0.00023	0.00007	0.00315	0.00382		
	3rd week	0.002762	0.00007	0.00002	0.00269	0.00291		
	4th week	0.002288	0.00015	0.00005	0.00211	0.00256		
Ni	1st week	0.00665	0.00098	0.00031	0.0055	0.0082	22.988	0.000 (HS)
	2nd week	0.003438	0.00198	0.00063	0.00083	0.00614		
	3rd week	0.00292	0.00011	0.00003	0.00275	0.00305		
	4th week	0.00497	0.00010	0.00003	0.00481	0.00515		
Cr	1st week	0.000336	0.00008	0.00002	0.00022	0.00044	21.902	0.000 (HS)
	2nd week	0.000204	0.00006	0.00002	0.00013	0.00031		
	3rd week	0.000376	0.00004	0.00001	0.00029	0.00042		
	4th week	0.000359	0.00002	0.00001	0.00033	0.00038		
Fe	1st week	0.5072	0.06324	0.019998	0.44	0.632	69.073	0.000 (HS)
	2nd week	0.2768	0.037938	0.011997	0.24	0.36		
	3rd week	0.29705	0.036262	0.011467	0.2275	0.344		
	4th week	0.2256	0.046464	0.014693	0.152	0.304		

Table 5: Concentration (µg/ml) A5

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Al	1st week	0.01065	0.002008	0.000635	0.0064	0.0142	172.348	0.000 (HS)
	2nd week	0.001929	0.000310	0.000098	0.00143	0.00241		
	3rd week	0.002224	0.000036	0.000011	0.00217	0.00229		
	4th week	0.002416	0.000182	0.000058	0.00204	0.00261		
Ni	1st week	0.00811	0.000504	0.000159	0.0072	0.0088	423.728	0.000 (HS)
	2nd week	0.002038	0.000608	0.000192	0.00109	0.00312		
	3rd week	0.002704	0.000077	0.000025	0.00261	0.00284		
	4th week	0.004426	0.000260	0.000082	0.00392	0.00465		
Cr	1st week	0.000284	0.000058	0.000018	0.0002	0.00038	4.375	0.000 (HS)
	2nd week	0.000262	0.000032	0.000010	0.00019	0.00031		
	3rd week	0.000284	0.000020	0.000006	0.00025	0.00031		
	4th week	0.000318	0.000009	0.000003	0.00031	0.00033		
Fe	1st week	0.4448	0.070975	0.022444	0.312	0.512	11.696	0.000 (HS)
	2nd week	0.3056	0.035337	0.011175	0.264	0.368		
	3rd week	0.3081	0.031827	0.010065	0.264	0.36		
	4th week	0.352	0.084664	0.026773	0.232	0.488		

Table 6: Concentration (µg/ml) B1

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Ni	1st week	0.0061	0.00018	0.00006	0.0058	0.0064	207.337	0.000 (HS)
	2nd week	0.00608	0.00019	0.00006	0.0058	0.0064		
	3rd week	0.00741	0.00018	0.00006	0.0071	0.0077		
	4th week	0.00783	0.00023	0.00007	0.0075	0.0082		
Cr	1st week	0.000406	0.00003	0.00001	0.00036	0.00045	74.711	0.000 (HS)
	2nd week	0.00044	0.00002	0.00001	0.00041	0.00049		
	3rd week	0.00052	0.00002	0.00001	0.00049	0.00055		
	4th week	0.000572	0.00004	0.00001	0.00051	0.00061		
Fe	1st week	0.4696	0.108848	0.034421	0.24	0.576	10.330	0.000 (HS)
	2nd week	0.4208	0.1398	0.044209	0.28	0.624		
	3rd week	0.568	0.122086	0.038607	0.424	0.744		
	4th week	0.2954	0.058606	0.018533	0.2	0.36		

Table 7: Concentration (µg/ml) B2

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Ni	1st week	0.0067	0.00015	0.00005	0.0065	0.0069	87.088	0.000 (HS)
	2nd week	0.00626	0.00022	0.00007	0.0059	0.0066		
	3rd week	0.00766	0.00028	0.00009	0.0072	0.0081		
	4th week	0.00766	0.00028	0.00009	0.0074	0.0083		
Cr	1st week	0.000368	0.00004	0.00001	0.00031	0.00044	75.926	0.000 (HS)
	2nd week	0.000413	0.00002	0.00001	0.00039	0.00045		
	3rd week	0.00052	0.00002	0.00001	0.00049	0.00055		
	4th week	0.000556	0.00004	0.00001	0.00049	0.00061		
Fe	1st week	0.3472	0.051873	0.016404	0.264	0.44	30.972	0.000 (HS)
	2nd week	0.28	0.069128	0.02186	0.192	0.384		
	3rd week	0.61625	0.085399	0.027006	0.456	0.7145		
	4th week	0.4387	0.112637	0.035619	0.32	0.632		

Table 8: Concentration (µg/ml) B3

Ions	Duration	Descriptive Statistics (N= 10)					Group Comparison	
		Mean	S.D.	S.E.	Min.	Max.	F-test	p-value
Ni	**1st week**	0.0063	0.00018	0.00006	0.006	0.0066	129.085	0.000 (HS)
	2nd week	0.00686	0.00016	0.00005	0.0066	0.0071		
	3rd week	0.00738	0.00018	0.00006	0.0071	0.0076		
	4th week	0.00776	0.00019	0.00006	0.0075	0.0081		
Cr	**1st week**	0.000348	0.00003	0.00001	0.00031	0.0004	252.605	0.000 (HS)
	2nd week	0.000438	0.00002	0.00001	0.00041	0.00047		
	3rd week	0.000528	0.00001	0.00000	0.00051	0.00055		
	4th week	0.00058	0.00002	0.00001	0.00055	0.00061		
Fe	**1st week**	0.2848	0.071475	0.022602	0.184	0.384	101.890	0.000 (HS)
	2nd week	0.2944	0.056669	0.01792	0.24	0.432		
	3rd week	0.76265	0.063254	0.020003	0.644	0.8375		
	4th week	0.4496	0.084899	0.026847	0.344	0.568		

Table 9: Values of group A

Group A	E-corr	I-core
A1	-159.4	2.42
A2	-144.5	2.29
A3	-164.1	3.02
A4	-157.3	1.38
A5	-195.2	1.96

Table 10: Value of group B

Group B	E-corr	I-core
B1	-111	1.92
B2	-125.7	3.7
B3	-12	4.96