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Interferences of Different Concentration of Detergent Solution on Glucose Assays in Clinical Chemistry

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ABSTRACT

Background: The pre-analytical stage is so complex that a mistake at any step often becomes apparent in the analytical or post-analytical phase. Many medical laboratorians do not carefully evaluate the suitability of the pre-analytical challenges in laboratory testing especially in the area of washing test tubes by various forms of detergent in the wash-up room.

Objective: This study was then aimed at the effect of different concentration of detergent solution on glucose assay in the clinical chemistry laboratory.

Materials and Methods: A total of twenty (20) subjects who visited a clinic in Chemical pathology unit, Federal Medical Centre, Owo were randomly recruited for this case study. The recruited subjects were analyzed for glucose assays normally and also at a different concentration of the detergent solution.

Results: There was a statistically significant difference between glucose value with no dilution and detergent stock solution while others were not significantly different from normal statistically even though there is slightly different in their mean values.

Conclusion: Prevention of pre-analytical errors from detergent solution remains an ongoing problem in the wash-up room and ultimately affects the ability of clinical laboratories to produce accurate results. Therefore, it is important to establish close working relationships with laboratory attendants working in the wash-up room and also, developing a surveillance program should be considered to prevent such occurrences.

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Keywords: Detergent, Glucose oxidase, Serial dilution, Glucose assays.

1. Introduction:

An appropriate pre-analytical handling is essential because the influence of this step on laboratory services could not be overlooked. Proper washing of test tubes, blood collection and timely preparation of samples for laboratory processing are critical pre-analytical steps required for the integrity of laboratory reports (Bowen and Remaley, 2014).

However, many medical laboratorians do not carefully evaluate the suitability of the pre-analytical challenges in laboratory testing especially in the area of washing test tubes by various forms of detergent in the wash-up room.

Detergents are widely used in clinical biochemistry, cell biology or molecular biology. Cell lysis, protein solubilization, protein crystallization or reduction of background staining in blotting experiments is just a few of numerous applications (Caligur, 2008; AppliChem, 2008). Detergents can be classified for instance according to their chemical structure stating their constituent polar and non-polar group (glucosides, alkyl ionic detergents, polyoxyethylene alcohols, bile salts, sulphonates etc.), the charge character (anionic, cationic, zwitterionic = amphoteric and non-ionic) or simply whether they are mild or strong in terms of their

ability to solubilize and/or to denature proteins (AppliChem, 2008).

They all have in common that they are soluble amphipathic (amphiphilic) compounds, with both lipophilic (hydrophobic, non-polar) and lipophobic (hydrophilic, polar) sites within one molecule. The key to detergent function is an amphipathic structure. All detergents are characterized as containing a hydrophilic “head” region and a hydrophobic “tail” region (see Figure 1) (Caligur, 2008). Therefore, these structural characteristics allow detergents to aggregate in aqueous media.

At a sufficiently high concentration (called Critical Micellar Concentration (CMC) value), the polar hydrophilic region of each molecule is oriented toward the polar solute (water) while the hydrophobic regions are grouped together to form thermodynamically stable micelles with hydrophobic cores. The hydrophobic core region of the detergent micelle associates with the hydrophobic surfaces of proteins and results in soluble protein-detergent complexes (See Figure 2) (Garavito and Ferguson-Miller, 2001; Caligur, 2008; AppliChem, 2008). This value is specific to each detergent and different factors like temperature, chemical structure, salt concentration and pH value may influence it (Garavito and Ferguson-Miller, 2001).

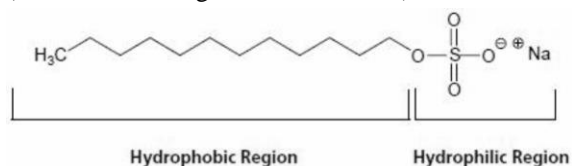


Figure 1. The structure of the anionic detergent sodium dodecyl sulphate (SDS), showing the hydrophilic and hydrophobic regions. (Source: Caligur, 2008)

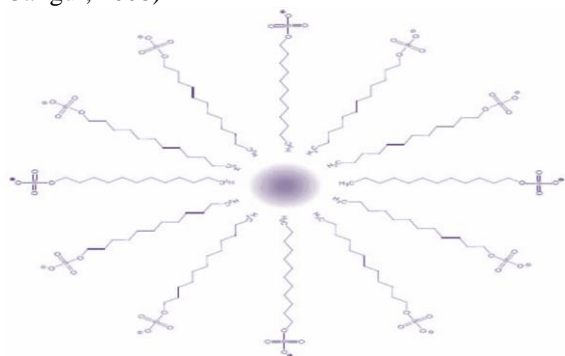


Figure 2. A simple illustration of a sodium dodecyl sulphates micelle. (Source: Caligur, 2008)

Although other authors have described the effects of endogenous substances on clinical assay results; the effects/impact of blood collection tube additives and components had also been well documented (Bowen and Remaley, 2014). But a large

proportion, possibly a majority, of errors in laboratory medicine occurs in the pre-analytical phase of the testing process (Plebani, 2006; Livesey et al., 2008).

Accurate laboratory testing requires an understanding of the complex interactions between collection devices and blood specimens, samples analyzed and assay reagents and detergent washing solution used in recycling test tubes for subsequent analysis especially in developing countries. In this review, we discuss how different concentration of the detergent solution can alter glucose test results, with an emphasis on inappropriate rinsing recycled test tubes which may give different dilution detergent solution and possibly, way to minimize their effects on clinical chemistry assays.

2. Materials and Methods:

2.1. Subjects:

A total of twenty (20) subjects (both males and females) aged between 18 – 60 years from Chemical pathology unit, Federal Medical Centre, Owo was randomly recruited for this case study after obtaining an approval from the Federal Medical Centre (FMC) Joint Ethics Review Committee (FMC/OW/380/VOL.XXXVI/197) and obtained written informed consent (approved by the FMC Ethics committee) from each subject.

2.2. Inclusion and Exclusion Criteria:

Apparently healthy volunteers were randomly recruited for the study. Subjects who did not give their consent were excluded from the study; so also subjects that were less than 18years of age.

2.3. Blood Collection:

A Blood sample was obtained from each subject by applying a tourniquet around the arm above the elbow.

The antecubital fossa was disinfected with a 70 percent alcohol-soaked swab. Five milliliters (5mls) of venous blood was collected from each subject using aseptic procedure after 12 hours fast with all bio-safety precautions (Ray et al., 2006). The blood was dispensed into fluoride oxalate bottle and plasma was separated from the blood after centrifuging at 2000g/m for 10minutes in standard bench centrifuge to obtain plasma required for glucose estimation.

2.4. Chemical Substance of Detergent used:

Linear Alkyl Benzene sulfonate (LABS), Sodium Tripolyphosphate (STPP), Sodium Carbonate (Na₂CO₃), and Sodium Sulphate (Na₂SO₄) were dissolved in distilled water at the concentration of 10% (w/v) as working detergent solution (This would be referred to as ‘neat’ which is equal to 10g/dl).

2.5. Preparation of Serial dilution for working detergent solution:

A dilution series is a succession of step dilutions, each with the same dilution factor, where the diluted material of the previous step is used to make the subsequent dilution (Ochei and Kolhatkar, 2008).

To make a dilution series (Figure 3), use the following formulas:

$$\text{Move Volume} = \text{Final Volume} / (\text{DF} - 1)$$

$$\text{Diluent Volume} = \text{Final Volume} - \text{Move Volume}$$

$$\text{Total Mixing Volume} = \text{Diluent Volume} + \text{Move Volume}$$

Working detergent solution was prepared as explained above at the concentration of 10% (w/v) ('neat' which is equal to 10g/dl). Thus, 10mls of this solution was pipetted into a test tube and was serially diluted at 1:10, 1:100 and 1:1000 as shown below. Then, five test tubes were arranged for each sample and 10uL of the sample was pipetted into all the test tubes. The detergent working solution was not added to the first test tube while 10uL of neat, 1:10, 1:100 and 1:1000 working detergent solution was added respectively to other arranged test tubes. This procedure was carried out for all the subjects recruited for the case study except blank and glucose standard.

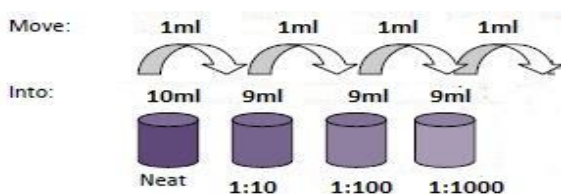


Figure 3. A dilution series.

2.6. Glucose Assay:

Glucose estimation was done using glucose oxidase method (enzymatic colorimetric) as described by Chessbrough, (2009).

3. Statistical Analysis:

A statistical package for social scientist (SPSS) 17.0 was used for the analysis of the data appropriately. The level of significance was taken at 95% confidence interval and P value less than 0.05 was considered significant.

4. Results:

Table 1 shows mean and Standard deviation of glucose estimated at different dilution detergent solution and no dilution (normal). In table 2, there is clear statistically significant difference between glucose value with no dilution (normal) and neat (detergent stock solution) while others are not significantly different from normal statistically even

though, there is slightly different in their mean values. Representation of mean glucose estimated against their respective dilution was shown in Figure 4.

Table 1: Mean and Standard deviation of glucose estimated at different dilution detergent solution and no dilution (normal)

Reagent	Dilution	Mean	Std. Deviation	N
Glucose	Neat	136.74	34.54	20
	1:10	109.57	37.48	20
	1:100	103.88	35.67	20
	1:1000	103.24	32.52	20
	Normal	103.09	26.35	20
	Total	111.30	35.33	100

Table 2: Contrast tests between glucose estimated at different dilution detergent solution and with no dilution (normal)

Contrast	Value of Contrast	Std. Error	t	df	p-Value
Glucose Neat Vs Normal	33.66	10.60	3.17	95	0.002*
1:10 Vs Normal	3.24	5.30	0.61	95	0.543
1:100 Vs Normal	1.19	15.90	0.08	95	0.940
1:1000 Vs Normal	0.02	1.06	0.02	95	0.988

* Significant at $p \geq 0.05$

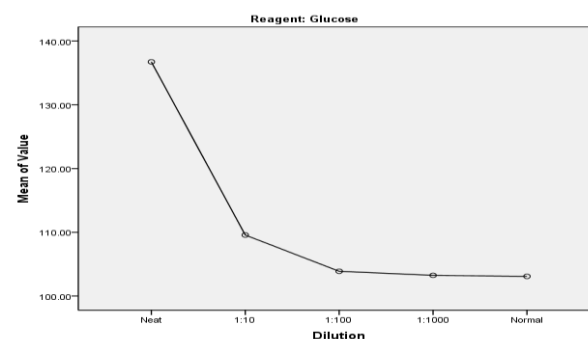


Figure 4. Representation of mean glucose estimated against their respective dilution

5. Discussion:

There are vast amounts of data, especially concerning the interaction of proteins with detergents using various techniques for more than three decades (Tsuge et al., 1984). All detergents are characterized as containing a hydrophilic region and a hydrophobic region which is the key to their function. (Caligur, 2008). These unique structural features allow detergents to aggregate in aqueous media. The

hydrophobic core region of the detergent micelle associates with the hydrophobic surfaces of proteins and results in soluble protein-detergent complexes (Garavito and Ferguson-Miller, 2001; Caligur, 2008).

The pre-analytical stage is so complex that a mistake at any step often becomes apparent in the analytical or post-analytical phase (Çuhadar, 2013). Total quality could be defined as the guarantee of a correctly performed activity throughout the total testing process (Delanghe and Speeckaert, 2014), providing accurate, precise and reliable medical diagnosis and efficient patient care in the medical laboratory. Various sampling methods, inappropriate specimen transport and detergent washing solution used in recycling test tubes for subsequent analysis can cause obvious pre-analytical errors. It is thus mandatory to focus on the pre-analytical phase in order to improve the reliability of test results (Delanghe and Speeckaert, 2014). This study was then aimed at the effect of different concentration of detergent solution on glucose assay in the clinical chemistry laboratory.

Table 1 shows increase mean glucose estimated at different serial dilution detergent solution from neat through 1:1000 dilution in comparing to mean of glucose obtained with no dilution. Similarly, there is clear statistically significant difference between glucose value with no dilution (normal) and neat (detergent stock solution) while others are not significantly different from normal statistically even though there is slightly different in their mean values. In contrast, Tsuge et al., 1984 demonstrated inactivation of glucose oxidase by the cationic detergent, while an anionic detergent did not produce measurable changes in the enzyme activity. Even though, this study could not ascertain the mechanism by which that glucose oxidase enzyme was protected against denaturation in different dilution of a detergent solution, activation of its activity might be due to zwitterionic nature of detergent used.

6. Conclusion and recommendations:

Prevention of pre-analytical errors from detergent solution remains an ongoing problem in the wash-up room and ultimately affects the ability of clinical laboratories to produce accurate results. Because it is not possible for laboratory personnel to assess the impact of their tubes on assay platforms because transparent residues other than the active ingredient may be present on the surface of test tubes and are not soluble in the cleaning solvent. It is thus important that they establish close working relationships with laboratory attendants working in the wash-up room for appropriate washing and

rinsing. Also, developing a surveillance program should be also considered to quickly identify problems of such and prevent it.

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The Jiang Periodic Table of Elements

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ABSTRACT

Using the stable number theory, Jiang's calculates the best electron configurations of the elements and not from experimental data (Chun-Xuan, 1988, Jiang, 1998; Jiang, 2002). Jiang makes the Jiang periodic table of the elements. In studying the stability of the many-body problem we suggest two principles (Jiang, 1981; Chun-Xuan, 1979; Jiang, 1985; Jiang, 1986; Jiang, 1988; Chun-Xuan, 1988, Jiang, 1998; Jiang, 2002).

(1) The prime number principle. A prime number is irreducible in the integers; it seems, therefore, natural to associate it with the most stable subsystem. We prove that 1, 3, 5, 7, 11, 23, 47 are the most stable primes.

(2) The symmetric principle. The most stable configuration of two prime numbers is then the stable symmetric system in nature. We prove that 2, 4, 6, 10, 14, 22, 46, 94 are the most stable even numbers. The stability can be defined as long life and existence in nature, and instability as short life or non-existence in nature.

In this paper by using the prime number principle and the symmetric principle we calculate the best electron configurations of the elements. Total quantum number and orbital quantum number determine the best electron configurations of the elements

Electron shells: $n=1 \quad 2 \quad 3 \quad 4 \quad 5 \quad 6\dots$
 $K \quad L \quad M \quad N \quad O \quad P\dots$

Electron subshells: $2(2l+1)=2 \quad 6 \quad 10 \quad 14 \quad 18 \quad 22\dots$
 $s \quad p \quad d \quad f \quad g \quad h\dots$

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Keywords: The Jiang periodic table, Table of Elements, electron configurations.

An atomic subshell that contains its full quota of electrons is said to be closed. A closed s subshell ($l = 0$) holds two electrons, a closed p subshell ($l = 1$) six electrons, a closed d subshell ($l = 2$) ten electrons, a closed f subshell ($l = 3$) fourteen electrons, these subshells are the most stable, a closed g subshell ($l = 4$) eighteen electrons is the most unstable. Using the symmetric principle it has been proved the $2(2l+1) = 2, 6, 10$ and 14 are stable and $2(2l+1) = 18$ is unstable. The s, p, d , and f subshells are stable and the g subshell is unstable (Jiang, 1985).



Table 1 shows the best electron configurations of the elements. From 1 to 92 of the atomic numbers every subshell is stable. It has been proved that the last stable element that occurs naturally is uranium with an atomic number of 92 and there are only 92 stable elements in nature. Since $5g$ subshell is unstable, the elements 93-110 are unstable. Since $5g$ is unstable, $6s, 6p, 6d, 6f, 6g$ and $6h$ subshells are unstable. Therefore, the elements 111-182 are unstable. Mendeleev electronic configuration of the elements is wrong (Scerri, 2007) to see table 3.

Using the $1s, 2s, 3s, 4s$ and $5s$ of table 1, we make the Jiang periodic table of elements with five periods. Table 2 shows the relationship between the outermost subshell electron configurations and the Jiang periodic table. The Jiang periodic table reflects the order in which atomic orbitals are filled. The s orbitals are filled in the two rows. The p orbitals are filled in the six rows. The d orbitals are filled in the ten rows. The f orbitals are filled in the fourteen rows. The g orbitals are filled in the eighteen rows.

Table 1: *The Best Electron Configuration of the Elements*

Z	Sym	K			L			M			N				O		
		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	5g	
1	H	1															
2	He	2															
3	Li	2	1														
4	Be	2	2														
5	B	2	2	1													
6	C	2	2	2													
7	N	2	2	3													
8	O	2	2	4													
9	F	2	2	5													
10	Ne	2	2	6													
11	Na	2	2	6	1												
12	Mg	2	2	6	2												
13	Al	2	2	6	2	1											
14	Si	2	2	6	2	2											
15	P	2	2	6	2	3											
16	S	2	2	6	2	4											
17	Cl	2	2	6	2	5											
18	Ar	2	2	6	2	6											
19	K	2	2	6	2	6	1										
20	Ca	2	2	6	2	6	2										
21	Sc	2	2	6	2	6	3										
22	Ti	2	2	6	2	6	4										
23	V	2	2	6	2	6	5										
24	Cr	2	2	6	2	6	6										
25	Mn	2	2	6	2	6	7										
26	Fe	2	2	6	2	6	8										
27	Co	2	2	6	2	6	9										
28	Ni	2	2	6	2	6	10										
29	Cu	2	2	6	2	6	10	1									
30	Zn	2	2	6	2	6	10	2									
31	Ga	2	2	6	2	6	10	2	1								
32	Ge	2	2	6	2	6	10	2	2								
33	As	2	2	6	2	6	10	2	3								
34	Se	2	2	6	2	6	10	2	4								



35	Br	2	2	6	2	6	10	2	5							
36	Kr	2	2	6	2	6	10	2	6							
37	Rb	2	2	6	2	6	10	2	6	1						
38	Sr	2	2	6	2	6	10	2	6	2						
39	Y	2	2	6	2	6	10	2	6	3						
40	Zr	2	2	6	2	6	10	2	6	4						
41	Nb	2	2	6	2	6	10	2	6	5						
42	Mo	2	2	6	2	6	10	2	6	6						
43	Tc	2	2	6	2	6	10	2	6	7						
44	Ru	2	2	6	2	6	10	2	6	8						
45	Rh	2	2	6	2	6	10	2	6	9						
46	Pd	2	2	6	2	6	10	2	6	10						
47	Ag	2	2	6	2	6	10	2	6	10	1					
48	Cd	2	2	6	2	6	10	2	6	10	2					
49	In	2	2	6	2	6	10	2	6	10	3					
50	Sn	2	2	6	2	6	10	2	6	10	4					
51	Sb	2	2	6	2	6	10	2	6	10	5					
52	Te	2	2	6	2	6	10	2	6	10	6					
53	I	2	2	6	2	6	10	2	6	10	7					
54	Xe	2	2	6	2	6	10	2	6	10	8					
55	Cs	2	2	6	2	6	10	2	6	10	9					
56	Ba	2	2	6	2	6	10	2	6	10	10					
57	La	2	2	6	2	6	10	2	6	10	11					
58	Ce	2	2	6	2	6	10	2	6	10	12					
59	Pr	2	2	6	2	6	10	2	6	10	13					
60	Nd	2	2	6	2	6	10	2	6	10	14					
61	Pm	2	2	6	2	6	10	2	6	10	14	1				
62	Sm	2	2	6	2	6	10	2	6	10	14	2				
63	Eu	2	2	6	2	6	10	2	6	10	14	2	1			
64	Gd	2	2	6	2	6	10	2	6	10	14	2	2			
65	Tb	2	2	6	2	6	10	2	6	10	14	2	3			
66	Dy	2	2	6	2	6	10	2	6	10	14	2	4			
67	Ho	2	2	6	2	6	10	2	6	10	14	2	5			
68	Er	2	2	6	2	6	10	2	6	10	14	2	6			
69	Tm	2	2	6	2	6	10	2	6	10	14	2	6	1		
70	Yb	2	2	6	2	6	10	2	6	10	14	2	6	2		
71	Lu	2	2	6	2	6	10	2	6	10	14	2	6	3		
72	Hf	2	2	6	2	6	10	2	6	10	14	2	6	4		
73	Ta	2	2	6	2	6	10	2	6	10	14	2	6	5		
74	W	2	2	6	2	6	10	2	6	10	14	2	6	6		
75	Re	2	2	6	2	6	10	2	6	10	14	2	6	7		
76	Os	2	2	6	2	6	10	2	6	10	14	2	6	8		
77	Ir	2	2	6	2	6	10	2	6	10	14	2	6	9		
78	Pt	2	2	6	2	6	10	2	6	10	14	2	6	10		
79	Au	2	2	6	2	6	10	2	6	10	14	2	6	10	1	
80	Hg	2	2	6	2	6	10	2	6	10	14	2	6	10	2	
81	Tl	2	2	6	2	6	10	2	6	10	14	2	6	10	3	
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83	Bi	2	2	6	2	6	10	2	6	10	14	2	6	10	5	
84	Po	2	2	6	2	6	10	2	6	10	14	2	6	10	6	
85	At	2	2	6	2	6	10	2	6	10	14	2	6	10	7	
86	Rn	2	2	6	2	6	10	2	6	10	14	2	6	10	8	
87	Fr	2	2	6	2	6	10	2	6	10	14	2	6	10	9	



88	Ra	2	2	6	2	6	10	2	6	10	14	2	6	10	10	
89	Ac	2	2	6	2	6	10	2	6	10	14	2	6	10	11	
90	Th	2	2	6	2	6	10	2	6	10	14	2	6	10	12	
91	Pa	2	2	6	2	6	10	2	6	10	14	2	6	10	13	
92	U	2	2	6	2	6	10	2	6	10	14	2	6	10	14	
93	Np	2	2	6	2	6	10	2	6	10	14	2	6	10	14	1
94	Pu	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2
95	Am	2	2	6	2	6	10	2	6	10	14	2	6	10	14	3
96	Cm	2	2	6	2	6	10	2	6	10	14	2	6	10	14	4
97	Bk	2	2	6	2	6	10	2	6	10	14	2	6	10	14	5
98	Cf	2	2	6	2	6	10	2	6	10	14	2	6	10	14	6
99	Es	2	2	6	2	6	10	2	6	10	14	2	6	10	14	7
100	Fm	2	2	6	2	6	10	2	6	10	14	2	6	10	14	8
101	Md	2	2	6	2	6	10	2	6	10	14	2	6	10	14	9
102	No	2	2	6	2	6	10	2	6	10	14	2	6	10	14	10
103	Lr	2	2	6	2	6	10	2	6	10	14	2	6	10	14	11
104	Rf	2	2	6	2	6	10	2	6	10	14	2	6	10	14	12
105	Db	2	2	6	2	6	10	2	6	10	14	2	6	10	14	13
106	Sg	2	2	6	2	6	10	2	6	10	14	2	6	10	14	14
107	Bh	2	2	6	2	6	10	2	6	10	14	2	6	10	14	15
108	Hs	2	2	6	2	6	10	2	6	10	14	2	6	10	14	16
109	Mt	2	2	6	2	6	10	2	6	10	14	2	6	10	14	17
110	Ds	2	2	6	2	6	10	2	6	10	14	2	6	10	14	18



Table 2: *The Jiang periodic table of elements.*

Atomic Orbitals	Outermost Subshell electrons	1. Period	2. Period	3. Period	4. Period	5. Period
s	1 2	1 H 2 He	3 Li 4 Be	11 Na 12 Mg	29 Cu 30 Zn	61 Pm 62 Sm
p	1 2 3 4 5 6		5 B 6 C 7 N 8 O 9 F 10 Ne	13 Al 14 Si 15 P 16 S 17 Cl 18 Ar	31 Ga 32 Ge 33 As 34 Se 35 Br 36 Kr	63 Eu 64 Gd 65 Tb 66 Dy 67 Ho 68 Er
d	1 2 3 4 5 6 7 8 9 10	Stable elements		19 K 20 Ca 21 Sc 22 Ti 23 V 24 Cr 25 Mn 26 Fe 27 Co 28 Ni	37 Rb 38 Sr 39 Y 40 Zr 41 Nb 42 Mo 43 Tc 44 Ru 45 Rh 46 Pd	69 Tm 70 Yb 71 Lu 72 Hf 73 Ta 74 W 75 Re 76 Os 77 Ir 78 Pt
f	1 2 3 4 5 6 7 8 9 10 11 12 13 14	Stable elements			47 Ag 48 Cd 49 In 50 Sn 51 Sb 52 Te 53 I 54 Xe 55 Cs 56 Ba 57 La 58 Ce 59 Pr 60 Nd	79 Au 80 Hg 81 Tl 82 Pb 83 Bi 84 Po 85 At 86 Rn 87 Fr 88 Ra 89 Ac 90 Th 91 Pa 92 U
g	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18	Unstable elements				93 Np 94 Pu 95 Am 96 Cm 97 Bk 98 Cf 99 Es 100 Fm 101 Md 102 No 103 Lr 104 Rf 105 Db 106 Sg 107 Bh 108 Hs 109 Mt 110 Ds

Table 3: *Mendeleev electronic configuration of the elements*

Num.	Symbol	K	L	M	N				O				P				Q				
1. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
1	<u>H</u>	1																			
2	<u>He</u>	2																			
2. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
3	<u>Li</u>	2	1																		
4	<u>Be</u>	2	2																		
5	<u>B</u>	2	2	1																	
6	<u>C</u>	2	2	2																	
7	<u>N</u>	2	2	3																	
8	<u>O</u>	2	2	4																	
9	<u>F</u>	2	2	5																	
10	<u>Ne</u>	2	2	6																	
3. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
11	<u>Na</u>	2	2	6	1																
12	<u>Mg</u>	2	2	6	2																
13	<u>Al</u>	2	2	6	2	1															
14	<u>Si</u>	2	2	6	2	2															
15	<u>P</u>	2	2	6	2	3															
16	<u>S</u>	2	2	6	2	4															
17	<u>Cl</u>	2	2	6	2	5															
18	<u>Ar</u>	2	2	6	2	6															
4. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
19	<u>K</u>	2	2	6	2	6	..	1													
20	<u>Ca</u>	2	2	6	2	6	..	2													
21	<u>Sc</u>	2	2	6	2	6	1	2													
22	<u>Ti</u>	2	2	6	2	6	2	2													
23	<u>V</u>	2	2	6	2	6	3	2													
24	<u>Cr</u>	2	2	6	2	6	5	1													
25	<u>Mn</u>	2	2	6	2	6	5	2													
26	<u>Fe</u>	2	2	6	2	6	6	2													
27	<u>Co</u>	2	2	6	2	6	7	2													
28	<u>Ni</u>	2	2	6	2	6	8	2													
29	<u>Cu</u>	2	2	6	2	6	10	1													
30	<u>Zn</u>	2	2	6	2	6	10	2													
31	<u>Ga</u>	2	2	6	2	6	10	2	1												
32	<u>Ge</u>	2	2	6	2	6	10	2	2												
33	<u>As</u>	2	2	6	2	6	10	2	3												
34	<u>Se</u>	2	2	6	2	6	10	2	4												
35	<u>Br</u>	2	2	6	2	6	10	2	5												
36	<u>Kr</u>	2	2	6	2	6	10	2	6												
5. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
37	<u>Rb</u>	2	2	6	2	6	10	2	6	1									
38	<u>Sr</u>	2	2	6	2	6	10	2	6	2									
39	<u>Y</u>	2	2	6	2	6	10	2	6	1	..	2									
40	<u>Zr</u>	2	2	6	2	6	10	2	6	2	..	2									
41	<u>Nb</u>	2	2	6	2	6	10	2	6	4	..	1									
42	<u>Mo</u>	2	2	6	2	6	10	2	6	5	..	1									
43	<u>Tc</u>	2	2	6	2	6	10	2	6	6	..	1									
44	<u>Ru</u>	2	2	6	2	6	10	2	6	7	..	1									
45	<u>Rh</u>	2	2	6	2	6	10	2	6	8	..	1									



46	<u>Pd</u>	2	2	6	2	6	10	2	6	10									
47	<u>Ag</u>	2	2	6	2	6	10	2	6	10	..	1									
48	<u>Cd</u>	2	2	6	2	6	10	2	6	10	..	2									
49	<u>In</u>	2	2	6	2	6	10	2	6	10	..	2	1								
50	<u>Sn</u>	2	2	6	2	6	10	2	6	10	..	2	2								
51	<u>Sb</u>	2	2	6	2	6	10	2	6	10	..	2	3								
52	<u>Te</u>	2	2	6	2	6	10	2	6	10	..	2	4								
53	<u>I</u>	2	2	6	2	6	10	2	6	10	..	2	5								
54	<u>Xe</u>	2	2	6	2	6	10	2	6	10	..	2	6								
6. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
55	<u>Cs</u>	2	2	6	2	6	10	2	6	10	..	2	6	1					
56	<u>Ba</u>	2	2	6	2	6	10	2	6	10	..	2	6	2					
57	<u>La</u>	2	2	6	2	6	10	2	6	10	..	2	6	1	..	2					
58	<u>Ce</u>	2	2	6	2	6	10	2	6	10	2	2	6	2					
59	<u>Pr</u>	2	2	6	2	6	10	2	6	10	3	2	6	2					
60	<u>Nd</u>	2	2	6	2	6	10	2	6	10	4	2	6	2					
61	<u>Pm</u>	2	2	6	2	6	10	2	6	10	5	2	6	2					
62	<u>Sm</u>	2	2	6	2	6	10	2	6	10	6	2	6	2					
63	<u>Eu</u>	2	2	6	2	6	10	2	6	10	7	2	6	2					
64	<u>Gd</u>	2	2	6	2	6	10	2	6	10	7	2	6	1	..	2					
65	<u>Tb</u>	2	2	6	2	6	10	2	6	10	9	2	6	2					
66	<u>Dy</u>	2	2	6	2	6	10	2	6	10	10	2	6	2					
67	<u>Ho</u>	2	2	6	2	6	10	2	6	10	11	2	6	2					
68	<u>Er</u>	2	2	6	2	6	10	2	6	10	12	2	6	2					
69	<u>Tm</u>	2	2	6	2	6	10	2	6	10	13	2	6	2					
70	<u>Yb</u>	2	2	6	2	6	10	2	6	10	14	2	6	2					
71	<u>Lu</u>	2	2	6	2	6	10	2	6	10	14	2	6	1	..	2					
72	<u>Hf</u>	2	2	6	2	6	10	2	6	10	14	2	6	2	..	2					
73	<u>Ta</u>	2	2	6	2	6	10	2	6	10	14	2	6	3	..	2					
74	<u>W</u>	2	2	6	2	6	10	2	6	10	14	2	6	4	..	2					
75	<u>Re</u>	2	2	6	2	6	10	2	6	10	14	2	6	5	..	2					
76	<u>Os</u>	2	2	6	2	6	10	2	6	10	14	2	6	6	..	2					
77	<u>Ir</u>	2	2	6	2	6	10	2	6	10	14	2	6	7	..	2					
78	<u>Pt</u>	2	2	6	2	6	10	2	6	10	14	2	6	9	..	1					
79	<u>Au</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	1					
80	<u>Hg</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2					
81	<u>Tl</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	1				
82	<u>Pb</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	2				
83	<u>Bi</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	3				
84	<u>Po</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	4				
85	<u>At</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	5				
86	<u>Rn</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	6				
7. Period		1s	2s	2p	3s	3p	3d	4s	4p	4d	4f	5s	5p	5d	5f	6s	6p	6d	6f	7s	7p
87	<u>Fr</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	6	1	
88	<u>Ra</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	6	2	
89	<u>Ac</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	6	1	..	2	
90	<u>Th</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	..	2	6	2	..	2	
91	<u>Pa</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	2	2	6	1	..	2	
92	<u>U</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	3	2	6	1	..	2	
93	<u>Np</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	4	2	6	1	..	2	
94	<u>Pu</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	6	2	6	2	
95	<u>Am</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	7	2	6	2	
96	<u>Cm</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	7	2	6	1	..	2	



97	<u>Bk</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	9	2	6	2
98	<u>Cf</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	10	2	6	2
99	<u>Es</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	11	2	6	2
100	<u>Fm</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	12	2	6	2
101	<u>Md</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	13	2	6	2
102	<u>No</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	2
103	<u>Lr</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	1	..	2
104	<u>Rf</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	2	..	2
105	<u>Db</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	3	..	2
106	<u>Sg</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	4	..	2
107	<u>Bh</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	5	..	2
108	<u>Hs</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	6	..	2
109	<u>Mt</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	7	..	2
110	<u>Uun</u>	2	2	6	2	6	10	2	6	10	14	2	6	10	14	2	6	9	..	1

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Jiang's Function $J_{n+1}(\omega)$ in Prime Distribution

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ABSTRACT

Jiang's define that prime equations

$$f_1(P_1, \dots, P_n), \dots, f_k(P_1, \dots, P_n) \quad (5)$$

are polynomials (with integer coefficients) irreducible over integers, where P_1, \dots, P_n are all prime. If Jiang's function $J_{n+1}(\omega) = 0$ then $J_{n+1}(\omega) \neq 0$ (has finite prime solutions. If $J_{n+1}(\omega) \neq 0$ then there are infinitely many primes P_1, \dots, P_n such that are primes. We obtain a unit prime formula in prime distribution

$$\begin{aligned} \pi_{k+1}(N, n+1) &= |\{P_1, \dots, P_n \leq N : f_1, \dots, f_k \text{ are } k \text{ primes}\}| \\ &= \prod_{i=1}^k (\deg f_i)^{-1} \times \frac{J_{n+1}(\omega) \omega^k}{n! \phi^{k+n}(\omega)} \frac{N^n}{\log^{k+n} N} (1 + o(1)). \end{aligned} \quad (8)$$

Jiang's function is accurate sieve function. Using Jiang's function, we prove about 600 prime theorems Jiang's function provides proofs of the prime theorems which are simple enough to understand and accurate enough to be useful.

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INTRODUCTION

"Mathematicians have tried in vain to discover some order in the sequence of prime numbers but we have every reason to believe that there are some mysteries which the human mind will never penetrate".

Leonhard Euler

"It will be another million years, at least, before we understand the primes".

Paul Erdős

Suppose that Euler totient function

$$\phi(\omega) = \prod_{2 \leq P} (P-1) = \infty \text{ as } \omega \rightarrow \infty, \quad (1)$$

where $\omega = \prod_{2 \leq P} P$ is called primordial.

Suppose that $(\omega, h_i) = 1$, where $i = 1, \dots, \phi(\omega)$. We have prime equations

$$P_1 = \omega n + 1, \dots, P_{\phi(\omega)} = \omega n + h_{\phi(\omega)} \quad (2)$$

where $n = 0, 1, 2, \dots$.

(2) is called infinitely many prime equations (IMPE). Every equation has infinitely many prime solutions. We have

$$\pi_{h_i} = \sum_{\substack{P_i \leq N \\ P_i \equiv h_i \pmod{\omega}}} 1 = \frac{\pi(N)}{\phi(\omega)}(1 + o(1)), \quad (3)$$

where π_{h_i} denotes the number of primes $P_i \leq N$ in $P_i = \omega n + h_i$, $n = 0, 1, 2, \dots$, $\pi(N)$ the number of primes less than or equal to N .

We replace sets of prime numbers by IMPE. (2) is the fundamental tool for proving the prime theorems in prime distribution.

Let $\omega = 30$ and $\phi(30) = 8$. From (2) we have eight prime equations

$$P_1 = 30n + 1, P_2 = 30n + 7, P_3 = 30n + 11, P_4 = 30n + 13, P_5 = 30n + 17, \\ P_6 = 30n + 19, P_7 = 30n + 23, P_8 = 30n + 29, n = 0, 1, 2, \dots \quad (4)$$

Every equation has infinitely many prime solutions.

THEOREM. We define that prime equation

$$f_1(P_1, \dots, P_n), \dots, f_k(P_1, \dots, P_n) \quad (5)$$

are polynomials (with integer coefficients) irreducible over integers, where P_1, \dots, P_n are primes. If Jiang's function $J_{n+1}(\omega) = 0$ then (5) has finite prime solutions. If $J_{n+1}(\omega) \neq 0$ then there exist infinitely many primes P_1, \dots, P_n such that each f_k is prime.

PROOF. Firstly, we have Jiang's function

$$J_{n+1}(\omega) = \prod_{3 \leq P} [(P-1)^n - \chi(P)], \quad (6)$$

where $\chi(P)$ is called sieve constant and denotes the number of solutions for the following congruence

$$\prod_{i=1}^k f_i(q_1, \dots, q_n) \equiv 0 \pmod{P}, \quad (7)$$

where $q_1 = 1, \dots, P-1, \dots, q_n = 1, \dots, P-1$.

$J_{n+1}(\omega)$ denotes the number of sets of P_1, \dots, P_n prime equations such that $f_1(P_1, \dots, P_n), \dots, f_k(P_1, \dots, P_n)$ are prime equations. If $J_{n+1}(\omega) = 0$ then (5) has finite prime solutions. If $J_{n+1}(\omega) \neq 0$ using $\chi(P)$ we sift out from (2) prime equations which cannot be represented P_1, \dots, P_n , then residual prime equations of (2) are P_1, \dots, P_n prime equations such that $f_1(P_1, \dots, P_n), \dots, f_k(P_1, \dots, P_n)$ are prime equations. Therefore, we prove that there exist infinitely many primes P_1, \dots, P_n such that $f_1(P_1, \dots, P_n), \dots, f_k(P_1, \dots, P_n)$ are primes.

Secondly, we have the best asymptotic formula

$$\pi_{k+1}(N, n+1) = |\{P_1, \dots, P_n \leq N : f_1, \dots, f_k \text{ are } k \text{ primes}\}| \\ = \prod_{i=1}^k (\deg f_i)^{-1} \times \frac{J_{n+1}(\omega) \omega^k}{n! \phi^{k+n}(\omega)} \frac{N^n}{\log^{k+n} N} (1 + o(1)). \quad (8)$$

(8) is called a unit prime formula in prime distribution. Let $n = 1, k = 0$, $J_2(\omega) = \phi(\omega)$. From (8) we have prime number theorem

$$\pi_1(N, 2) = |\{P_1 \leq N : P_1 \text{ is prime}\}| = \frac{N}{\log N} (1 + o(1)). \quad (9)$$

Number theorists believe that there are infinitely many twin primes, but they do not have a rigorous proof of this old conjecture by any method. All the prime theorems are conjectures except the prime number theorem, because they do not prove that prime equations have infinitely many prime solutions. We prove the following conjectures by

this theorem.

Example 1. Twin primes $P, P + 2$ (300BC).

From (6) and (7) we have Jiang's function

$$J_2(\omega) = \prod_{3 \leq P} (P-2) \neq 0.$$

Since $J_2(\omega) \neq 0$ in (2) exist infinitely many P prime equations such that $P + 2$ is a prime equation. Therefore, we prove that there are infinitely many primes P such that $P + 2$ is prime.

Let $\omega = 30$ and $J_2(30) = 3$. From (4) we have three P prime equations

$$P_3 = 30n + 11, \quad P_5 = 30n + 17, \quad P_8 = 30n + 29.$$

From (8) we have the best asymptotic formula

$$\begin{aligned} \pi_2(N, 2) &= |\{P \leq N : P + 2 \text{ prime}\}| = \frac{J_2(\omega)\omega}{\phi^2(\omega)} \frac{N}{\log^2 N} (1 + o(1)) \\ &= 2 \prod_{3 \leq P} \left(1 - \frac{1}{(P-1)^2}\right) \frac{N}{\log^2 N} (1 + o(1)). \end{aligned}$$

In 1996, we proved twin primes conjecture (Chun-xuan & Bingui, 1996)

Remark. $J_2(\omega)$ denotes the number of P prime equations, $\frac{\omega}{\phi^2(\omega)} \frac{N}{\log^2 N} (1 + o(1))$ the number of solutions of primes for every P prime equation.

Example 2. Even Goldbach's conjecture $N = P_1 + P_2$. Every even number $N \geq 6$ is the sum of two primes.

From (6) and (7) we have Jiang's function

$$J_2(\omega) = \prod_{3 \leq P} (P-2) \prod_{P|N} \frac{P-1}{P-2} \neq 0.$$

Since $J_2(\omega) \neq 0$ as $N \rightarrow \infty$ in (2) exist infinitely many P_1 prime equations such that $N - P_1$ is a prime equation.

Therefore, we prove that every even number $N \geq 6$ is the sum of two primes.

From (8) we have the best asymptotic formula

$$\begin{aligned} \pi_2(N, 2) &= |\{P_1 \leq N, N - P_1 \text{ prime}\}| = \frac{J_2(\omega)\omega}{\phi^2(\omega)} \frac{N}{\log^2 N} (1 + o(1)) \\ &= 2 \prod_{3 \leq P} \left(1 - \frac{1}{(P-1)^2}\right) \prod_{P|N} \frac{P-1}{P-2} \frac{N}{\log^2 N} (1 + o(1)). \end{aligned}$$

In 1996, we proved even Goldbach's conjecture (Chun-xuan & Bingui, 1996)

Example 3. Prime equations $P, P + 2, P + 6$.

From (6) and (7) we have Jiang's function

$$J_2(\omega) = \prod_{5 \leq P} (P-3) \neq 0,$$

$J_2(\omega)$ is denotes the number of P prime equations such that $P + 2$ and $P + 6$ are prime equations. Since $J_2(\omega) \neq 0$ in (2) exist infinitely many P prime equations such that $P + 2$ and $P + 6$ are prime equations.

Therefore, we prove that there are infinitely many primes P such that $P + 2$ and $P + 6$ are primes.

Let $\omega = 30$, $J_2(30) = 2$. From (4) we have two P prime equations

$$P_3 = 30n + 11, \quad P_5 = 30n + 17.$$

From (8) we have the best asymptotic formula

$$\pi_3(N, 2) = |\{P \leq N : P + 2, P + 6 \text{ are primes}\}| = \frac{J_2(\omega)\omega^2}{\phi^3(\omega)} \frac{N}{\log^3 N} (1 + o(1)).$$

Example 4. Odd Goldbach's conjecture $N = P_1 + P_2 + P_3$. Every odd number $N \geq 9$ is the sum of three primes.

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P} (P^2 - 3P + 3) \prod_{P|N} \left(1 - \frac{1}{P^2 - 3P + 3}\right) \neq 0.$$

Since $J_3(\omega) \neq 0$ as $N \rightarrow \infty$ in (2) exist infinitely many pairs of P_1 and P_2 prime equations such that $N - P_1 - P_2$ is a prime equation. Therefore, we prove that every odd number $N \geq 9$ is the sum of three primes.

From (8) we have the best asymptotic formula

$$\begin{aligned} \pi_2(N, 3) &= \left| \{P_1, P_2 \leq N : N - P_1 - P_2 \text{ prime}\} \right| = \frac{J_3(\omega)\omega}{2\phi^3(\omega)} \frac{N^2}{\log^3 N} (1 + o(1)). \\ &= \prod_{3 \leq P} \left(1 + \frac{1}{(P-1)^3}\right) \prod_{P|N} \left(1 - \frac{1}{P^3 - 3P + 3}\right) \frac{N^2}{\log^3 N} (1 + o(1)). \end{aligned}$$

Example 5. Prime equation $P_3 = P_1P_2 + 2$.

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P} (P^2 - 3P + 2) \neq 0$$

$J_3(\omega)$ denotes the number of pairs of P_1 and P_2 prime equations such that P_3 is a prime equation. Since $J_3(\omega) \neq 0$ in (2) exist infinitely many pairs of P_1 and P_2 prime equations such that P_3 is a prime equation.

Therefore, we prove that there are infinitely many pairs of primes P_1 and P_2 such that P_3 are prime.

From (8) we have the best asymptotic formula

$$\pi_2(N, 3) = \left| \{P_1, P_2 \leq N : P_1P_2 + 2 \text{ prime}\} \right| = \frac{J_3(\omega)\omega}{4\phi^3(\omega)} \frac{N^2}{\log^3 N} (1 + o(1)).$$

Note. $\deg(P_1P_2) = 2$.

Example 6(Heath-Brown, 2001). Prime equation $P_3 = P_1^3 + 2P_2^3$.

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P} [(P-1)^2 - \chi(P)] \neq 0,$$

where $\chi(P) = 3(P-1)$ if $2^{\frac{P-1}{3}} \equiv 1 \pmod{P}$; $\chi(P) = 0$ if $2^{\frac{P-1}{3}} \not\equiv 1 \pmod{P}$; $\chi(P) = P-1$ otherwise.

Since $J_3(\omega) \neq 0$ in (2) there are infinitely many pairs of P_1 and P_2 prime equations such that P_3 is a prime equation. Therefore, we prove that there are infinitely many pairs of primes P_1 and P_2 such that P_3 are prime.

From (8) we have the best asymptotic formula

$$\pi_2(N, 3) = \left| \{P_1, P_2 \leq N : P_1^3 + 2P_2^3 \text{ prime}\} \right| = \frac{J_3(\omega)\omega}{6\phi^3(\omega)} \frac{N^2}{\log^3 N} (1 + o(1)).$$

Example 7 (Friedlander & Iwaniec, 1998). Prime equation $P_3 = P_1^4 + (P_2 + 1)^2$.

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P} [(P-1)^2 - \chi(P)] \neq 0$$

where $\chi(P) = 2(P-1)$ if $P \equiv 1 \pmod{4}$; $\chi(P) = 2(P-3)$ if $P \equiv 1 \pmod{8}$; $\chi(P) = 0$ otherwise.

Since $J_3(\omega) \neq 0$ in (2) there are infinitely many pairs of P_1 and P_2 prime equations such that P_3 is a prime equation. Therefore, we prove that there are infinitely many pairs of primes P_1 and P_2 such that P_3 are prime.

From (8) we have the best asymptotic formula

$$\pi_2(N, 3) = |\{P_1, P_2 \leq N : P_3 \text{ prime}\}| = \frac{J_3(\omega)\omega}{8\phi^3(\omega)} \frac{N^2}{\log^3 N} (1 + o(1)).$$

Example 8 (Szemerédi, 1975; Furstenberg, 1977; Gowers, 2001; Kra, 2006; Green & Tao, 2008; Tao, 2006; Green, 2007). Arithmetic progressions consisting only of primes. We define the arithmetic progressions of length k .

$$P_1, P_2 = P_1 + d, P_3 = P_1 + 2d, \dots, P_k = P_1 + (k-1)d, (P_1, d) = 1. \quad (10)$$

From (8) we have the best asymptotic formula

$$\begin{aligned} \pi_2(N, 2) &= |\{P_1 \leq N : P_1, P_1 + d, \dots, P_1 + (k-1)d \text{ are primes}\}| \\ &= \frac{J_2(\omega)\omega^{k-1}}{\phi^k(\omega)} \frac{N}{\log^k N} (1 + o(1)). \end{aligned}$$

If $J_2(\omega) = 0$ then (10) has finite prime solutions. If $J_2(\omega) \neq 0$ then there are infinitely many primes P_1 such that P_2, \dots, P_k are primes.

To eliminate d from (10) we have

$$P_3 = 2P_2 - P_1, \quad P_j = (j-1)P_2 - (j-2)P_1, 3 \leq j \leq k.$$

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P < k} (P-1) \prod_{k \leq P} (P-1)(P-k+1) \neq 0$$

Since $J_3(\omega) \neq 0$ in (2) there are infinitely many pairs of P_1 and P_2 prime equations such that P_3, \dots, P_k are prime equations. Therefore, we prove that there are infinitely many pairs of primes P_1 and P_2 such that P_3, \dots, P_k are primes.

From (8) we have the best asymptotic formula

$$\begin{aligned} \pi_{k-1}(N, 3) &= |\{P_1, P_2 \leq N : (j-1)P_2 - (j-2)P_1 \text{ prime}, 3 \leq j \leq k\}| \\ &= \frac{J_3(\omega)\omega^{k-2}}{2\phi^k(\omega)} \frac{N^2}{\log^k N} (1 + o(1)) = \frac{1}{2} \prod_{2 \leq P < k} \frac{P^{k-2}}{(P-1)^{k-1}} \prod_{k \leq P} \frac{P^{k-2}(P-k+1)}{(P-1)^{k-1}} \frac{N^2}{\log^k N} (1 + o(1)). \end{aligned}$$

Example 9. It is a well-known conjecture that one of $P, P+2, P+2^2$ is always divisible by 3. To generalize above to the k -primes, we prove the following conjectures. Let n be a square-free even number.

1. $P, P+n, P+n^2,$

where $3|(n+1)$.

From (6) and (7) we have $J_2(3) = 0$, hence one of $P, P+n, P+n^2$ is always divisible by 3.

2. $P, P+n, P+n^2, \dots, P+n^4,$

where $5|(n+b), b = 2, 3$.

From (6) and (7) we have $J_2(5) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^4$ is always divisible by 5.

3. $P, P+n, P+n^2, \dots, P+n^6,$

where $7|(n+b), b = 2, 4$.

From (6) and (7) we have $J_2(7) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^6$ is always divisible by 7.

4. $P, P+n, P+n^2, \dots, P+n^{10},$

where $11|(n+b), b = 3, 4, 5, 9$.

From (6) and (7) we have $J_2(11) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^{10}$ is always divisible by 11.

5. $P, P+n, P+n^2, \dots, P+n^{12},$

where $13|(n+b), b = 2, 6, 7, 11$.

From (6) and (7) we have $J_2(13) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^{12}$ is always divisible by 13.

6. $P, P+n, P+n^2, \dots, P+n^{16}$,

where $17|(n+b), b = 3, 5, 6, 7, 10, 11, 12, 14, 15$.

From (6) and (7) we have $J_2(17) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^{16}$ is always divisible by 17.

7. $P, P+n, P+n^2, \dots, P+n^{18}$,

where $19|(n+b), b = 4, 5, 6, 9, 16, 17$.

From (6) and (7) we have $J_2(19) = 0$, hence one of $P, P+n, P+n^2, \dots, P+n^{18}$ is always divisible by 19.

Example 10. Let n be an even number.

1. $P, P+n^i, i = 1, 3, 5, \dots, 2k+1$,

From (6) and (7) we have $J_2(\omega) \neq 0$. Therefore, we prove that there exist infinitely many primes P such that $P, P+n^i$ are primes for any k .

2. $P, P+n^i, i = 2, 4, 6, \dots, 2k$.

From (6) and (7) we have $J_2(\omega) \neq 0$. Therefore, we prove that there exist infinitely many primes P such that $P, P+n^i$ are primes for any k .

Example 11. Prime equation $2P_2 = P_1 + P_3$

From (6) and (7) we have Jiang's function

$$J_3(\omega) = \prod_{3 \leq P} (P^2 - 3P + 2) \neq 0.$$

Since $J_3(\omega) \neq 0$ in (2) there are infinitely many pairs of P_1 and P_2 prime equations such that P_3 is prime equations. Therefore, we prove that there are infinitely many pairs of primes P_1 and P_2 such that P_3 are prime.

From (8) we have the best asymptotic formula

$$\pi_2(N, 3) = \left| \{P_1, P_2 \leq N : P_3 \text{ prime}\} \right| = \frac{J_3(\omega)\omega}{2\phi^3(\omega)} \frac{N^2}{\log^3 N} (1 + o(1)).$$

In the same way, we can prove $2P_2^2 = P_3 + P_1$ which has the same Jiang's function.

Conclusion

Jiang's function is accurate sieve function. Using it we can prove any irreducible prime equations in prime distribution. There are infinitely many twin primes but we do not have a rigorous proof of this old conjecture by any method (Green, 2007). As strong as the numerical evidence may be, we still do not even know whether there are infinitely many pairs of twin primes (Iwaniec & Kowalski, 2004). All the prime theorems are conjectures except the prime number theorem, because they do not prove the simplest twin primes. They conjecture that the prime distribution is randomness (Heath-Brown, 2001; Friedlander & Iwaniec, 1998; Szemerédi, 1975; Furstenberg, 1977; Gowers, 2001; Kra, 2006; Green & Tao, 2008; Tao, 2006; Green, 2007; Iwaniec & Kowalski, 2004; Crandall & Pomerance, 2005; Green, 2006; Soundararajan, 2007; Granville, 1995), because they do not understand theory of prime numbers.

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Determination of Heavy Metal Impurities in Facial Makeup Cosmetic Products in Sudanese Markets, using Inductively Coupled Plasma – Mass Spectrometry (ICP –MS)



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Abstract: Recently facial makeup cosmetic products have become very popular among Sudanese women in spite of the fact that they might contain toxic materials including Pb, Hg, Cd, As, Ni and Zn, and their use as ingredients in cosmetics is prohibited as they cause skin problems. Owing to the absence of control standards of these products sold in Sudanese markets, the present study aimed at developing a method for the determination of these metals by inductively coupled plasma – mass spectrometry (ICP-MS). Eighty-six samples of different facial makeup cosmetic products were collected randomly from the local Sudanese markets. A microwave- assisted digestion with a mixture of concentrated nitric acid and hydrochloric acid was applied for sample preparation. The concentration of each metal was determined in triplicate by ICP-MS. For eye shadow, in a wide range of concentrations, all analyzed elements were detected at high levels in the imported samples, but Pb, Hg, and Cd were detected at low levels in the locally prepared not only eye shadow but also lipstick samples. Although Pb was present at low concentrations in all samples, locally prepared whitening creams, lipstick, and eye shadow showed highest concentration levels of 33.63 $\mu\text{g/g}$, 42.42 $\mu\text{g/g}$, and 52.02 $\mu\text{g/g}$, respectively. The highest concentration levels for Hg in locally prepared whitening creams and eye shadow samples were found to be 42.09 $\mu\text{g/g}$, and 21.30 $\mu\text{g/g}$, respectively. The highest concentration levels for Cd, however, were found to be 5.21 $\mu\text{g/g}$, 22.53 $\mu\text{g/g}$, and 7.06 $\mu\text{g/g}$, in imported lipstick, eye shadow, and local whitening creams, respectively. High concentration levels of Zn were not obtained in all analyzed samples except in the imported eye shadow, (72.46 $\mu\text{g/g}$). The determination of heavy metals in cosmetic products sold in Sudanese markets increase the needs to set laws to regulate their preparation locally and to set standards to control their quality and safety.

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1. Introduction:

Cosmetics can be defined as many preparations to be applied to the human body for the purpose of cleansing, beautifying, promoting, making attractive or altering the appearance without affecting the body's structure and function. They can also be used to repair or hide skin imperfections, to cleanse, adorn, protect and treat the human body (Al-Dayel et al., 2011). In Sudanese markets, there are plenty of cosmetic products which are prepared locally without any control. The most alarming issue in Sudan is the large number of people practicing skin-lightening techniques. Similar practices are also employed by black ladies in all over the world (Adebajo, 2002).

The challenging problem of the cosmetic industry is to design safe products that help dark- skin woman looks "Lighter". Facial attractiveness, however, is associated with positive evaluation by others, and many Sudanese ladies believe that with the lighter skin they are more competent. Interestingly, the level of education, social class, marital and employment status seem to have less influence on the skin lightening practice. The toxicity of metals and its curie are well documented (Chukwuma, 1997). Since the issue of heavy metals, as deliberate cosmetic ingredients have been addressed, attention is focused on the presence of these substances as impurities, (Health Canada, 2011).

Heavy metals are not intentionally added to cosmetics as constituent ingredients; they are simply trace impurities in the product and are thus not required to be listed on the labels (Reed, 2007). Minute traces may be carried into the product during preparation. The metals of primary toxic concern in cosmetics are: Pb, Hg, Cd, As, Ni and Zn. Dermal exposure is expected to be the most significant route for cosmetic products since the majority of cosmetics are applied on the skin.

Dermal absorption of heavy metals is fairly minimal, with the absorption of individual elements influenced by a number of factors including physical and chemical properties of the mixture, (Dwivedi, (1996). The present study was carried out to develop a method for the determination of heavy metal impurities in the facial makeup cosmetic products in the local market by inductively coupled plasma – mass spectrometry (ICP-MS), (Lee, et al., 2008).

2. Materials and Methods:

2.1. Sample Collection:

Eighty-six different samples, commonly used as facial makeup, were collected from the local markets in Khartoum, Khartoum North, and Omdurman cities and were classified into four different groups marked with different letters and numbers.

Lipstick 25 samples: 20 imported (L1 – L20) and 5-local (LL21 – LL25).

Face Powder 25 local samples: (P26 – P50).

Creams, 30 samples: 15 local skin – lightening creams (LC 51 – LC 59), 15 imported skin – lightening cream (C 66 – C 80) and 6 - imported (F&L 60 – F&L 65).

Eye Shadow: 6 samples (ES91 – ES 96).

2.2. Sample Digestion:

Strict care was taken to ensure complete decomposition of the sample and to avoid contamination. Ultra-pure acids and distilled deionized water were used, (Volpe, 2012). All plastic and glassware were washed many times with tap water, soaked in 5% HNO₃ solution for a minimum of 24 hours and then rinsed with deionized water. Solid samples were dried in an oven at 105°C to constant weight and then stored in desiccators. About 3.5g of dried sample was weighed accurately in a porcelain crucible and dry-ashed in a muffle furnace by a stepwise increase of temperature up to 550 °C, for one hour.

The ashed samples were digested with a few cubic centimeters of 1M nitric acid, evaporated nearly to dryness on a hot plate, in fuming hood, cooled, filtered through Whatman No 42, and were diluted up to the 100 cm³ mark in a volumetric flask. Samples such as cream and lotion, which could not be

conveniently processed by dry ashing, were wet digested, with a 4:1 mixture of nitric acid (65%) and perchloric acid (70-72%) on a hot plate in fuming hood, nearly to dryness by slowly increasing the temperature, for 2-3 hours because oily compounds are exothermic and burn with flame.

In case that brown or black color appeared then again the same procedure was repeated by adding the mixture of concentrated acids by slow and continuous heating until the evolution of white fumes (marking the end of digestion process) and then, nearly to dryness, alternatively, sample solutions acidified with concentrated HNO₃ and HCl, were digested in a programmed microwave. The solutions were allowed to cool and filtered into a 100 cm³ volumetric flask by Whatman No 42 and diluted up to the volumetric mark, (Al-Ashban, 2004).

Like the sample solutions, blank solutions were prepared similarly including the addition of all reagents but excluding the presence of the samples.

3. Heavy metal analysis:

Cosmetic sample solutions were analyzed for the determination of the concentration of Pb, Hg, Cd, As, Ni and Zn. Using Agilent 7700X ICP – MS instrument, (Lee, et al., 2008).

4. Results and Discussion:

Table 1 shows the concentration of heavy metals in, twenty samples of imported lipstick, indicating Pb, Cd and As concentration ranged from 4.18 µg/g to 42.42 µg/g, 0.00 µg/g to 12.00 µg/g and from 0.00 µg/g to 10.21 µg/g, respectively. However, the results of determination of the concentration of heavy metals in, five samples of local lipstick were shown in Table 2.

The concentration of Pb, Hg, Ni and Zn metals ranged from 11.45 µg/g to 29.0 µg/g, 2.35 µg/g to 9.24 µg/g, 0.16 µg/g to 9.41 µg/g and 1.00 µg/g to 10.23 µg/g respectively. Table 3 shows that the concentration of, Pb and Zn metals in twenty-five samples of face - powder ranged from 0.04 µg/g to 20.02 µg/g and from 2.47 µg/g to 9.52 µg/g, respectively.

Table 4 shows the concentration of heavy metal impurities of six fair & lovely samples (F&L), and nine samples of local skin-lightening cream (LC). The concentration of Pb in F&L and LC ranged from 2.71 µg/g to 7.27 µg/g and from as much as 14.49 µg/g to as much as 33.36 µg/g, respectively. The concentration of Zn ranged from 1.19 µg/g to 4.91 µg/g and from as much as 2.41 µg/g to as much as 11.04 µg/g, respectively.

Table 5 shows the concentration of heavy metals in fifteen samples of imported skin-lightening



cream (C). The concentration of Pb, Hg, and Zn metals ranged from 0.09 $\mu\text{g/g}$ to 4.49 $\mu\text{g/g}$, from 0.08 $\mu\text{g/g}$ to 6.64 $\mu\text{g/g}$ and from 1.19 $\mu\text{g/g}$ to 10.35 $\mu\text{g/g}$ respectively. Table 6 shows that the concentration of heavy metals, Pb, Hg, Cd, Ni and Zn, in six eye shadow samples ranged from 17.22 $\mu\text{g/g}$ to 52.02 $\mu\text{g/g}$, from 8.10 $\mu\text{g/g}$ to 21.30 $\mu\text{g/g}$, from 10.52 $\mu\text{g/g}$ to 22.53 $\mu\text{g/g}$, from 12.25 $\mu\text{g/g}$ to 73.94 $\mu\text{g/g}$ and from 9.90 $\mu\text{g/g}$ to 72.46 $\mu\text{g/g}$ respectively.

Lead and cadmium are well known as potentially harmful metals that have aroused considerable interest. Particularly, lead has been described as the most harmful environmental contaminant recognized in human civilization and has shown renal impairment in patients with methylmalonic aciduria and in the nervous system as stated in different reports linking it to a deficiency in cognitive functioning (Piccinini et al., 2013).

The metals analyzed in this study were not listed as ingredients in any of the products. Due to the lack of manufacturer testing and regulatory oversight, it is possible that the companies themselves were not even aware that their products were contaminated with these toxic metals (Nnorom, et al., 2005). These contaminants most likely entered into the cosmetic products when poor-quality ingredients were used. It is possible that the source of these toxic metals could have been from one or more of the inorganic base material used in manufacturing processes, (Omar, et al., 2001). In spite of the absence of strict control of cosmetic products, those of local lipstick (Table 2), face powder (Table 3) and imported skin- lightening cream (Table 5) samples were laden poisonous metals of Pb, Hg, Cd, As, Ni and Zn.

Similarly, imported lipstick (Table 1) and local skin – lightening cream (Table 4) samples revealed high levels of Pb, as well as Hg respectively. Clinically, Pb poisoning from use of Pb- based eye cosmetics is presented with abdominal cramped pain, encephalopathy (manifested as anxiety and irritability), and anemia (Chauhan, et al., 2010). In Asia, Africa, and the Middle East, they had been identified as suspected source of Pb exposure to the ocular system, (Guy, et al., 1999).

A recurrent theme in research on childhood Pb poisoning has been discovered time and again over the past four decades that Pb is toxic to the developing nervous system at levels previously thought to be safe. The observation that these facial cosmetics are used on neonates and by pregnant women should elicit concerns. Studies have reported that breastfeeding enhances the release of Pb from the bones and that Pb freely crosses the placenta, (Shannon, 2003).

Consequently, gestational Pb poisoning is not only harmful to the woman, but also to the developing fetus, invariably producing congenital Pb poisoning.

Epidemiological studies confirm an association between Pb exposure and prevalence of dental carries in school-age children, (Lansdown, 2001). The applications of local preparations containing Pb and Hg, in the treatment of ophthalmic infections and as an eye cleanser and in cosmetic have been identified as a major source of Pb intake in Africa, (Dwivedi, 1996).

The present study indicated that the use of facial cosmetics sold in Sudanese markets exposes users to low levels of heavy metals of which Pb, Cd, and Hg are of most toxicological concern. Understanding the consequences of low-level human Pb and Cd poisoning will depend on upon an accurate assessment of the pervasiveness of toxicity in the global population.

This will require that toxicity thresholds be determined as well as an understanding of the mechanisms underlying toxicity, (International Union of P.A.C, 1976). Education of parents and childcare workers regarding the risks of administering heavy metals-based substances to children and themselves needs to be incorporated into health and healthcare framework systems in developing nations, (Campbell, et al., 2000). Keeping in view their toxicology, the estimation of toxic metals in cosmetic products has prompted us to carry out this study.

Unfortunately, there are no current international standards for impurities like heavy metals in cosmetics except 20 $\mu\text{g/g}$ for Lead and 5 $\mu\text{g/g}$ for cadmium, (Al-Trabulsy et al., 2013). The Canadian regularity limits for certain metals in cosmetic products are 10 for Pb, 3 for As, Cd, and Hg.

Health Canada but FDA recently allows being not more than (1ppb) in cosmetic products (Brown, 2013). Hg is toxic and rapid absorbed through the skin and can affect people neurologically causing blurred vision or trouble walking. The use of mercury in skin creams have been well documented in other countries and should be avoided. The continuous use of cosmetic products contaminated with such heavy metals may, however, cause a slow release of these metals into the human body and cause harmful effects to the consumer over time. Extensive use of such products should be avoided until the solution is adequately addressed. Removal of heavy metals from personal care products after manufacture is not possible. Nevertheless, careful selection of raw materials that are almost free from heavy metals can improve the quality of the products and save the beauty of the environment.

Finally, this study had revealed that continuous use of these cosmetics could result in an increase in the trace metal levels in the human body beyond acceptable limits. Efforts should be made at

enlightening the users and the general public on the dangers involved.

Table 1: Concentration of heavy metals in imported lipstick samples collected from Sudanese markets

Sample ID	Avg. conc. of Pb (µg/g)	Avg. conc. of Hg (µg/g)	Avg. conc. of Cd (µg/g)	Avg. conc. of As (µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn (µg/g)
L1	9.83	0.43	0.11	ND	ND	2.09
L2	12.5	ND	2.05	ND	0.95	1.32
L3	14.28	0.06	5.21	0.08	ND	2.17
L4	11.04	ND	0.74	ND	ND	1.04
L5	21.04	ND	0.69	ND	ND	5.06
L6	10.03	ND	1.82	ND	1.95	0.04
L7	16.93	0.22	1.77	5.14	ND	0.86
L8	8.47	1.33	2.11	4.40	3.16	ND
L9	42.42	0.25	0.43	2.71	ND	1.07
L10	12.34	0.61	ND	10.21	ND	2.35
L11	15.25	1.44	ND	5.01	ND	ND
L12	4.97	2.04	7.26	ND	0.20	0.69
L13	16.18	6.02	ND	3.00	ND	0.23
L14	22.10	ND	4.05	0.53	ND	0.84
L15	4.18	ND	12.00	0.08	ND	ND
L16	12.67	1.09	5.62	ND	ND	ND
L17	19.11	0.88	4.33	ND	ND	0.94
L18	11.75	ND	ND	ND	0.58	0.51
L19	6.08	ND	ND	ND	0.24	ND
L20	10.18	0.25	0.57	0.79	ND	ND

Where: ND, not detected, and L, for lipstick.

Table 2: Concentration of heavy metals in local lipstick samples collected from Sudanese markets

Sample ID	Avg. conc. of Pb (µg/g)	Avg. conc. of Hg (µg/g)	Avg. conc. of Cd (µg/g)	Avg. conc. of As (µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn (µg/g)
L L21	23.29	3.07	4.02	ND	2.35	10.23
L L22	28.59	8.96	ND	ND	9.41	5.20
L L23	11.45	8.01	0.66	0.24	5.80	1.00
L L24	17.41	2.35	1.50	ND	0.59	3.84
L L25	29.00	9.24	6.43	ND	0.16	7.47

Where ND, not detected, and LL, local lipstick.

Table 3: Concentration of heavy metals in face powder samples collected from local Sudanese markets

Sample ID	Avg. conc. of Pb (µg/g)	Avg. conc. of Hg (µg/g)	Avg. conc. of Cd (µg/g)	Avg. conc. of As (µg/g)	Avg. conc. of Ni (µg/g)	Avg. conc. of Zn (µg/g)
P26	2.15	1.73	0.52	ND	0.84	4.30
P27	0.04	0.30	1.50	ND	1.32	6.23
P28	16.20	ND	0.37	ND	0.42	2.47
P29	8.64	2.10	0.80	0.51	0.08	9.52
P30	3.25	ND	0.53	0.09	ND	5.81
P31	14.07	1.08	2.06	1.21	ND	7.04
P32	16.31	ND	0.73	ND	1.68	2.47
P33	8.12	ND	3.11	0.84	0.92	5.88
P34	2.48	1.06	0.64	ND	1.30	3.30
P35	6.07	0.94	0.39	ND	0.37	2.56
P36	3.37	ND	0.87	0.24	ND	7.11
P37	9.51	ND	1.25	ND	0.89	4.38
P38	0.73	0.05	ND	ND	1.24	6.01
P39	2.04	1.06	ND	1.07	0.51	2.67
P40	20.02	2.11	0.56	1.25	ND	7.81
P41	5.47	ND	0.43	0.06	ND	3.22
P42	6.13	ND	1.72	ND	2.50	6.30
P43	10.48	ND	0.84	ND	0.77	9.21
P44	8.09	ND	ND	ND	1.80	8.63
P45	1.55	0.77	ND	ND	0.08	5.00
P46	2.93	2.01	ND	ND	0.85	9.52
P47	0.05	ND	1.20	0.54	1.35	3.30
P48	7.15	ND	0.61	ND	0.87	2.61
P49	4.28	1.09	0.72	ND	0.99	6.82
P50	6.54	ND	1.46	ND	2.01	7.43

Where: ND; not detected and P face powder.

Table 4: Concentration of heavy metals in local skin-lightening cream samples (preparation) collected from Sudanese markets.

Sample ID	Avg. conc. of Pb ($\mu\text{g/g}$)	Avg. conc. of Hg ($\mu\text{g/g}$)	Avg. conc. of Cd ($\mu\text{g/g}$)	Avg. conc. of As ($\mu\text{g/g}$)	Avg. conc. of Ni ($\mu\text{g/g}$)	Avg. conc. of Zn ($\mu\text{g/g}$)
LC51	28.90	14.62	7.06	0.04	ND	2.41
LC52	14.49	14.10	ND	0.19	2.27	11.04
LC53	33.63	20.08	ND	0.05	0.54	2.92
LC54	17.13	42.09	1.09	0.96	ND	6.86
LC55	19.30	24.50	0.22	ND	0.88	1.18
LC56	30.63	16.05	ND	ND	1.36	3.20
LC57	23.68	21.63	2.79	0.29	1.26	6.27
LC58	17.20	13.02	ND	1.02	2.01	8.21
LC59	28.36	13.98	2.66	ND	ND	6.01
F&L 60	3.10	2.93	0.06	0.01	1.82	3.76
F&L 61	2.71	4.01	0.08	ND	0.65	7.63
F&L 62	3.80	2.51	ND	ND	0.27	2.40
F&L 63	7.27	1.67	0.03	0.05	0.51	5.16
F&L 64	6.51	2.04	0.09	ND	1.44	1.19
F&L 65	2.94	3.98	0.61	0.03	2.04	4.91

Where, ND not, detected, F&L fair & lovely and LC, local skin- lightening creams (local preparation).

Table 5: Concentration of heavy metals in imported skin - lightening cream samples collected from Sudanese markets

Sample ID	Avg. conc. of Pb ($\mu\text{g/g}$)	Avg. conc. of Hg ($\mu\text{g/g}$)	Avg. conc. of Cd ($\mu\text{g/g}$)	Avg. conc. of As ($\mu\text{g/g}$)	Avg. conc. of Ni ($\mu\text{g/g}$)	Avg. conc. of Zn ($\mu\text{g/g}$)
C66	1.04	2.93	0.06	0.01	1.82	3.67
C67	0.53	4.01	0.08	ND	0.65	7.63
C68	0.09	2.51	ND	ND	0.27	2.40
C69	0.41	1.67	0.03	ND	0.51	5.16
C70	2.37	2.04	0.09	0.05	1.44	1.19
C71	0.34	3.98	0.61	ND	2.04	4.91
C72	2.73	2.86	0.14	0.03	1.12	4.14
C73	3.44	0.08	ND	0.15	ND	10.35
C74	0.65	2.21	ND	ND	3.09	4.40
C75	2.47	1.08	0.77	ND	2.23	3.11
C76	1.07	6.64	0.80	ND	0.91	6.20

C77	0.18	3.21	ND	ND	1.55	8.62
C78	4.49	1.84	ND	ND	8.21	1.36
C79	1.55	0.96	0.21	ND	7.35	6.24
C80	2.53	0.88	1.50	ND	7.12	4.58

Where, ND, not detected, and C, for skin lightening creams (imported).

Table 6: Concentration of heavy metals in different brands of Eye Shadow samples collected from Sudanese market.

Sample ID	Avg. conc. of Pb ($\mu\text{g/g}$)	Avg. conc. of Hg ($\mu\text{g/g}$)	Avg. conc. of Cd ($\mu\text{g/g}$)	Avg. conc. of As ($\mu\text{g/g}$)	Avg. conc. of Ni ($\mu\text{g/g}$)	Avg. conc. of Zn ($\mu\text{g/g}$)
ES 91	24.48	12.73	10.52	0.07	73.94	14.30
ES 92	33.06	21.30	11.37	0.17	54.37	61.23
ES 93	40.07	19.21	18.72	ND	68.42	72.46
ES 94	17.22	8.10	22.53	ND	12.81	9.90
ES 95	52.02	9.74	12.13	ND	17.66	19.85
ES 96	29.95	11.12	10.56	ND	12.25	32.8

Where, ND: not detected, ES; eye shadow.

5. Conclusion:

Almost all imported and locally prepared facial makeup cosmetic samples were found to be contaminated with heavy metals. Results of ICP–MS analysis showed that the concentration ranged from 2.70 $\mu\text{g/g}$ to 52.02 $\mu\text{g/g}$ and from 1.09 $\mu\text{g/g}$ to 72.46 $\mu\text{g/g}$ for lead and zinc respectively, however, the concentration ranged from 11.04 $\mu\text{g/g}$ to 22.53 $\mu\text{g/g}$ and 73.94 $\mu\text{g/g}$ for Cd, As, and Ni, respectively. Moreover, the concentration of Hg, determined by using ICP-MS technique as well, ranged from 0.00 $\mu\text{g/g}$ to 22.53 $\mu\text{g/g}$.

6. Recommendation:

Cosmetic products should be frequently analyzed for safety purposes, not only for toxic metals but also for preservatives, colors, and any other additives. Urgent efforts are needed for the establishment of limits of toxic metals, permissible preservatives, and colors to use in cosmetic products in Sudanese markets.

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Resolution of Seizures in A Child with Dravet Syndrome and Immunoglobulin G Subclasses Deficiency Treated with Valproic Acid, Intravenous Immunoglobulin, and Vagal Nerve Stimulator

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ABSTRACT: Severe myoclonic epilepsy in infants, also known as Dravet syndrome, is a rare refractory form of epilepsy and its control requires a combination of several antiepileptic drugs. We describe a child who had Dravet syndrome unresponsive to a combination of drugs. Work-up for repeated infections revealed immunoglobulin G subclasses deficiency. Administration of intravenous immunoglobulin in addition to valproic acid did not control epilepsy but followed by Vagal Nerve Stimulator, resolution of seizures was sustained.

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1. Introduction:

Conventional antiepileptic drugs did not achieve seizure control in a child with severe myoclonic epilepsy; a combination of valproic acid, intravenous immunoglobulin for immunoglobulin G subclasses deficiency, and Vagal Nerve Stimulator achieved resolution of seizures. This novel combined approach (pharmacological, immunological and surgical) needs further evaluation in a subset of refractory severe myoclonic epilepsy.

2. Case Presentation:

This 8-year-old girl presented at the age of 4 years with a history of daily seizures since early infancy and recurrent episodes of prolonged febrile status epilepticus for more than 30 minutes necessitating multiple hospital admissions. Her seizures consisted of a combination of daily myoclonic fits, frequent focal, generalized tonic, and generalized tonic-clonic events upon febrile illness often ending up in prolonged status epilepticus. She was the product of a full-term pregnancy and a cesarean delivery with no perinatal complications.

She had borderline delayed neurodevelopmental milestones for age upon presentation and a difficult social interaction. Physical exam was unremarkable. Regular blood tests and thyroid functions test were non-revealing. Magnetic resonance imaging of the brain was normal. Multiple EEG recordings had frequent generalized spike waves and polyspike waves as well as multifocal abnormalities. A genetic blood test revealed a novel mutation c.5195C>T/p. Pro

1732Leu of the SCN1 (alpha subunit of the Type I voltage-gated sodium channel) gene that was not present in her parent's blood. Multiple medications were initiated including phenobarbital, valproic acid, topiramate, levetiracetam, lamotrigine, clonazepam, and clobazam, either as monotherapy or in different combinations, to no avail. The drugs did achieve therapeutic blood levels. The parents followed a strict regimen of adherence to medications and medical visits. Unfortunately, stiripentol was not available in our country.

Her neurodevelopmental delay and behavioral problems became more prominent. She was placed in a special school to meet her educational needs.

As she was noted to have very frequent febrile illnesses with accompanying seizures), the immune deficiency was suspected. Work up for immune deficiency revealed low levels of IgG2 (0.822 g/l), IgG3 (0.17g/l) and borderline IgG4 (<0.0691g/l), with the normal reference values being (1.06-6.1g/l), (0.18-1.63g/l) and (0.04-2.30g/l) for each respectively. Total IgG, IgA, and IgM levels were all normal. Upon these findings, the patient was kept only on Valproic acid (40 mg/kg/day) and was given a course of 2g/kg IV IgG (Octagam 10%-Octapharma AG, Lachen, Switzerland) over 5 days repeatedly, every month, for a total course of 6 months. Towards the end of the immunoglobulin course, the seizures were still refractory.

The decision then was to insert a vagal nerve stimulator (VNS- CyberonicsR, Houston, Texas). She underwent a smooth and uneventful surgery and was started on a gradual escalating program till

reaching the following parameters: a pulse width of 250 microseconds, a frequency of 20 Hz, a time on for 30 sec, a time off for 5min and an amplitude of 1mA. At that time, more than 50% of seizures had resolved and the febrile events had decreased from monthly to every 4 months.

Currently, the patient is seizure-free for more than 2 years. She is still on Valproic acid and under the same VNS parameters except for a time off for 1.8 min and amplitude of 2 mA. EEG continues to show frequent generalized high voltage and multifocal discharges. Her behavior has improved markedly and she has now better cognitive and scholastic performance with improved social interaction.

3. Discussion:

Dravet syndrome is a severe form of epilepsy with a great majority having a mutation in SCN1A gene, which encodes a voltage-gated sodium channel (Kwong et al., 2012). SCN1A-related seizure disorders are inherited in an autosomal dominant manner. A proband with an SCN1A-related seizure disorder may have an inherited or de novo mutation. The natural history of SCN1A-related seizure disorders is strongly influenced by seizure phenotype ranging from simple febrile seizures to severe myoclonic epilepsy of infancy.

Seizures in Dravet syndrome respond poorly to commonly used anti-epilepsy drugs. Initial results of clinical trials with newer medications (topiramate, levetiracetam), or with ketogenic diet appear promising (Chiron & Dulac, 2011). So far the best clinical response appears to derive from a combination of valproic acid, clobazam, and stiripentol (Al-Baradie, 2013). With this triple combination, both the frequency and the duration of seizures were reduced together with a decrease in the number of episodes of status epilepticus (Thanh et al., 2002). Stiripentol displays anti-epileptic effect by its effect on an alpha-3 subunit of the GABA-A receptors (Brigo & Storti, 2014). Recent studies have confirmed its efficacy as an add-on medication in Dravet syndrome (Inoue et al., 2009).

Stiripentol was granted an Orphan Designation for the treatment of Dravet syndrome in the US in 2008; however, the drug is still not FDA approved and is not available in a large part of the world, including Lebanon. A systematic review showed only 2 randomized clinical trials evaluating the use of stiripentol (total of 64 children) with findings of the significantly higher proportion of participants having 50% or greater reduction in seizure frequency in the stiripentol group compared with placebo (Inoue et al., 2009). Our patient's seizures were pharmaco-resistant. Thus, a more

aggressive intervention was needed to decrease the seizures and secondarily delay progressive cognitive and mental deterioration (Van Rijckevorsel, 2006).

Concomitant administration of VNS and immunoglobulins in our patient in addition to valproic acid mono pharmacotherapy resulted in resolution of seizures.

As recurrent acute illness seemed to be an important precipitating factor for the development of seizures and status epilepticus in our patient, the evaluation of underlying immunodeficiency was warranted. However, a clear relation between immunodeficiency and Dravet has not yet been established and the literature concerning the subject seems scarce (Plebani et al., 1987). Young patients with IgG subclasses deficiency tend to develop recurrent infections as was noted in our young girl (Plebani et al., 1987).

Therefore administering IV immunoglobulins becomes an integral part of antiepileptic treatment in subjects with immunodeficiency and epilepsy (Berger, 2008). Several reports have dealt with immunological abnormalities in epileptic patients, especially low levels of immunoglobulins (Villani & Avanzini, 2002; Quek et al., 2012; Engelen et al., 1994; Nieto et al., 2000; Geva-Dayan et al., 2012; Geng et al., 2011). At the same time, and interestingly enough, IV immunoglobulin therapy has shown to be beneficial for some patients with certain types of intractable epilepsy, whether as an immune modulator or as a direct neuromodulator as has been proved recently (Villani & Avanzini, 2002; Quek et al., 2012; Engelen et al., 1994; Nieto et al., 2000; Geva-Dayan et al., 2012; Geng et al., 2011). Thus, IV IgG has a dual beneficial therapeutic role: the first being for immunodeficiency diseases and the second for some types of intractable epilepsies.

On the other hand, the beneficial role of VNS in children with intractable epilepsy is well established (Elliott et al., 2011; Kuba et al., 2009; Cersósimo et al., 2011; Zamponi et al., 2011). Data regarding VNS in patients with Dravet syndrome is still scarce. Emerging laboratory evidence suggests that VNS, while restraining inflammatory cytokine production in the peripheral nervous system, also exerts a significant CNS neuroprotective function against ischemic stroke injury (Jiang) by inhibiting the apoptosis and oxidant stress responses associated with such injury (Jiang et al., 2015).

It is unclear which of the above treatment played the major role in decreasing the seizures in our patient or whether there was synergism between the 3 modalities. IV immunoglobulin therapy could have helped to attenuate the seizures severity by the time the effect of the VNS started to work. Whether

the beneficial effect of the immuno-modulatory drugs like IV immunoglobulin therapy continues to work, long after its administration course is finished, is unknown. Therefore, IV immunoglobulin could have still helped our patient long after 6 months of the administration. However, it was clear that after the gradual escalation of the VNS parameters the seizures started to decrease markedly.

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Effect of Two Commonly used Insecticides On Some Physico-Chemical Parameters and Microbial Load of a Farm Soil

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Abstract: Pesticides are generally used in agriculture to eliminate un-wanted growths and reduce productivity cost with little concern for non-target organisms. Studies were carried out on the effect of two commonly used insecticides (common names-DD force & Scorpio) on some soil physicochemical parameters and microbial loads at recommended rate (x1.0) and twice the recommended rate (x2.0) over a period of 8 weeks. Generally, insecticides treatments resulted in soil pH reduction from 7.23 for the control soil to 5.98 for DD force and 7.03 for Scorpio. At recommended application rates (x1.0) there was a decrease in bacterial counts at week 0 (6 hours after treatment)-control soil = 6.90×10^6 cfu/ml; DD force = 3.09×10^6 cfu/ml and Scorpio = 3.09×10^6 cfu/ml. Thereafter, there was an increase in bacterial counts reaching a maximum at week 4. This was followed by a general decrease in bacterial counts. Bacteria counts in soils treated with DD force and Scorpio at twice the recommended rates (x2.0) were higher compared to counts obtained from the two insecticides treated at the recommended rates (x1.0). However, fungal counts from soils treated with the two insecticides at recommended rates (x1.0) were higher compared to counts obtained from soils treated at twice the recommended rates. A total of 55 bacterial species were isolated from the insecticide treated and control soils which are Bacillus sp (30.9%), Proteus sp (12.7%), Flavobacterium sp (14.5%), Pseudomonas sp. (1.8%), Lactobacillus sp (10.9%), Staphylococcus sp (10.9%), Antinomycetes sp (12.7%). A total of 20 fungi isolated were isolated from insecticides treated soil and control soil which includes Aspergillus sp (40%), Penicillium sp (20%), Rhizopus sp (20%).

To cite this article

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Keywords: Insecticides, Physico-chemical, microbial counts, treatment rates

1. Introduction:

The Food and Agricultural Organization (FAO, 2007) has defined pesticides as any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human and animal diseases, unwanted species of plants or animals, causing harm during or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood and wood products or animal feedstuffs, or substances that may be administered to animals for the control of insects, arachnids, or other pests in or on their bodies (FAO, 2007).

Many modern pesticides do not persist for long in the environment. They act quickly and are then degraded to non-toxic substances by environmental and microbial processes. This helps prevent their build-up in crops or non-target organisms. How

quickly a pesticide breaks down depends on its chemical properties, how much is applied and how it is distributed, as well as environmental factors such as temperature, moisture, pH and the availability of microorganisms (USEPA, 2013). Microorganisms such as bacteria and fungi are very important ecologically as decomposers in the soil (Taylor, 2002). Microorganisms are of prime importance in the soil recycling of elements essentially for biological processes and thus the maintenance of soil fertility.

Pesticides are often applied directly to the soil. They may also reach the soil through application to foliage via spray drift, run-off or wash-of vectors (Racke and Coats, 1990). Pesticides in soil partition between at least three phases: soil air, soil solution and soil rocks (Gunter and Joseph, 2013).

Insecticides are substances used to kill or control insects. They are used in agriculture,

medicine, and the industry. Nearly all insecticides have the potential to significantly alter the ecosystem; many are toxic to man and some may concentrate along the food chain (USEPA, 2014). A major concern to pesticide use is the changes they may have on microbial populations and activities of individual species of microorganisms (Bollag et al., 2002; Ayansina & Oso, 2006).

Microorganisms are of prime importance in the recycling of elements essential for many biological processes and thus maintenance of soil fertility. In this study, we set out to know the effect of two commonly used insecticides soil pH, microbial counts and isolations.

2. Materials and Methods:

2.1. Sampling and insecticide treatment:

Soil samples were collected from the Bowen University, Agric farm, sieved through 2.0mm sieve to remove stone and plant debris. One kilogram each was placed in plastic bowls and treated with the 2 insecticides (DD force-2,2-dichlorovinyl dimethyl phosphate & Scorpio-dimethoate + cypermethrin) at manufacturers recommended rate (x 1.0) and twice recommended rate (x 2.0). Recommended rates of application for the two insecticides was done by mixing 10.5ml of each in 100ml of de-ionized water per 1.0kg of the soil sample. Another bowl treated with 100ml de-ionized water only served as the control. Soil treatments were replicated. Soil samples were analyzed on a weekly basis over 8-week sampling periods – for soil pH, total microbial counts, and isolations.

2.2. Determination of soil pH:

Twenty grams from each of the soil treatments was mixed with 20ml of de-ionized water and stirred thoroughly in 100ml baker. The soil-water mixture was allowed to settle for 30 minutes with intermittent mixing and stirring. The coarse particles were allowed to settle and pH was determined using the pH meter (Jenway model 3540).

2.3. Microbial counts and Isolations:

One from each of the soils treated was mixed with 9ml sterile de-ionized water in a test tube to obtain 10⁻¹ dilution. Several dilutions were made to the 10⁻⁶ by pipetting 1ml from previous dilutions to fresh 9ml sterile de-ionized water.

One millilitre was pipette from each of the dilutions and transferred on to sterile Petri dishes. Nutrient agar (for bacterial growth) and potato dextrose agar (for fungal growth) was poured into the Petri dishes and allowed to set. The plates were replicated and labeled appropriately. Nutrient agar plates were incubated at 35°C for 24 hours while

potato dextrose agar plates were incubated at 25°C for 48 hours. Discrete colonies were counted from which estimation was done on the microbial counts from the treated and control soils. Microbial counts were done at week 0 (6 hours after initial treatments) and fortnightly over 8 weeks. Discrete colonies were sub-cultured on fresh sterile agar plates to obtain pure cultures representative of each treatment. Conventional identification steps included Gram's staining, catalase test, oxidase test, citrate test, sugar fermentation test, microscopic observation etc.

3. Results and Discussion:

Generally, soil treatment with the two insecticides resulted in a pH reduction from a mean value at 7.23 for the control soil to 5.98 for DD force and 7.03 for Scorpio Table 1. This result contrast Ayansina and Oso (2006) who reported that there were no significant changes in soil pH of soils treated with two different pesticides. The effectiveness of pesticides can be affected by soil pH, moisture contents and humidity, temperature, organic matter and the addition of organic amendments (Akobundu, 1987).

Compared to the untreated (control) soil, there was an initial decrease in mean bacterial counts in DD force treated soils at week 0 (6.90×10^6 cfu/ml to 3.09×10^6 cfu/ml) and for Scorpio treated soil (from 6.90×10^6 cfu/ml to 3.00×10^6 cfu/ml)-all at recommended rates. Thereafter, there was a relative increase in bacterial counts reaching a maximum (3.0×10^6 cfu/ml and 3.50×10^6 cfu/ml) at week 4 in DD force treated soils (Fig 1). A general decrease in bacterial counts was obtained in Scorpio treated soils as shown in Figure 2. Generally, bacterial counts in soils treated with DD force and Scorpio at twice the recommended rates (x 2) resulted in higher counts compared to those treated at recommended rates.

However, fungal counts were higher in soils treated at the recommended rates compared to those treated at twice the recommended rates. DD force treated soils at recommended rate resulted in mean fungal counts of 2.8×10^4 cfu/ml while at twice recommended rate mean fungal counts of 2.4×10^4 cfu/ml was obtained at week 0. Scorpio treated soil had 3.0×10^4 cfu/ml and 2.6×10^4 cfu/ml at recommended and twice recommended rates respectively (Tables 3 and 4). It has been shown that pesticides effect on soil microflora could be inhibitory, stimulatory or neutral (Gogotov, 1992; Bollag et al., 2002). Moorman et al. (2001) had reported that distribution in microbial counts, activities and species can be caused by application of pesticides at greater or higher concentrations than the recommended doses. A decline in microbial counts after each peak must have been due to the fact that

microbial populations that were tolerant of the treated pesticides were susceptible to the products of soil-pesticide interactions, which could have been possibly bactericidal or fungicidal (Taiwo and Oso, 1997).

A total of 55 bacterial species was isolated from the insecticide treated and control soils which comprise *Bacillus* sp. (30.9%), *Proteus* sp. (12.7%), *Flavobacterium* sp. (14.5%), *Pseudomonas* sp. (1.8%), *Lactobacillus* sp. (10.9%), and *Staphylococcus* sp. (10.9%), Actinomycetes (12.7%) as presented in Table 2. On the other hand, a total of 20 fungi was isolated and these includes *Aspergillus* sp (40%), *Penicillium* sp. (20%), and *Rhizopus* sp. (20%)-as shown also in Table 2. These microorganisms are mainly indigenous to the soil and are capable of metabolizing insecticides provided other environmental conditions are right Mervat, 2009).

Indiscriminate uses of insecticides, when applied to soils, can have several effects on soil microbial community. A major way of improving agricultural production to meet human demands is the use of insecticides to control insects. In achieving sustainable agricultural production, environmental implications of these insecticides should be considered and the miss-use of these insecticides should be discouraged due to damages to the soil ecosystems and environmental hazards.

Table 1: PH measurements from insecticide-treated soils and control soil

Treatment/Week	Week 0	Week 2	Week 4	Week 6	Week 8	Mean
DD Force x 1.0	6.2	5.4	6.4	6.1	5.9	6.00
DD Force x 2.0	5.6	5.0	5.4	5.2	5.4	5.98
Scorpio x 1.0	7.3	6.7	6.7	6.6	6.8	6.82
Scorpio x 2.0	7.4	7.0	6.9	6.8	6.8	7.03
Control	7.9	6.9	7.0	7.1	7.1	7.23

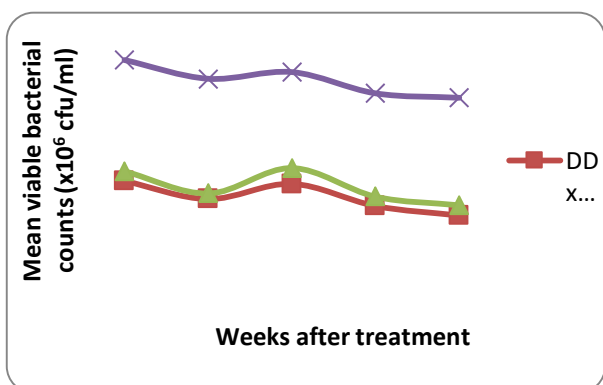


Figure 1: mean viable bacterial counts at recommended rate (x1.0) and twice recommended rate (x2.0) for the insecticide DD Force

Table 2: Microbial isolations from insecticide-treated soils and control soil

Microorganisms	Amount	%
Bacterial		
<i>Bacillus</i> sp	17	30.9
<i>Proteus</i> sp	7	12.7
<i>Flavobacterium</i> sp	8	14.5
<i>Lactobacillus</i> sp	6	10.9
<i>Staphylococcus</i> sp	6	10.9
<i>Pseudomonas</i> sp	1	1.8
Actinomycetes	7	12.7
Unidentified	3	5.5
Fungal		
<i>Aspergillus</i> sp	8	40
<i>Penicillium</i> sp	4	20
<i>Rhizopus</i> sp	4	20
Unidentified	4	20

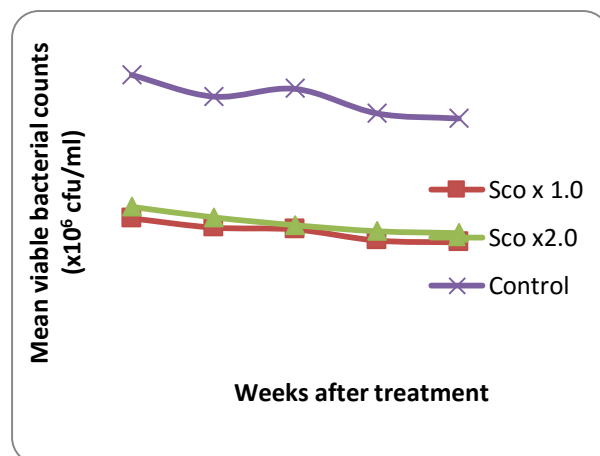


Figure 2: mean viable bacterial counts at recommended rate (x1.0) and twice recommended rate (x2.0) for the insecticide Scorpio

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Psycho-Analysis of The Cricketer Javed Miandad's Behavior and Thoughts During the Famous Sharjah Last Ball Sixer

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ABSTRACT

Objective: To study how people behave under stressful conditions and how Javed Miandad behaved and thought during the famous Sharjah last ball sixer.

Method: Besides literature search a survey was conducted with 126 participants from ages 18 to 59 with half of them males and half of them females. Only one survey question was included. The question was "If you were batting instead of Javed Miandad on the last ball and 4 runs were needed and it was the same match the final India vs Pakistan. You were 110 not out and a batsman of that caliber. What would you be feeling?" The study was conducted from September 1st, 2014 to September 30th, 2015 in the San Francisco Bay Area, California including participants from different parts of the world including Pakistan and India.

Results: The results came out with 73.8 % people saying they would be confident with a belief that they can hit a six, 31 % said they would be nervous or fearful while the rest had mix feelings.

Conclusion: The study concludes that the behavior under the stressful situation when millions of people are watching is independent of the situation but depends on the individual. Some people will react with confidence while other react in a different manner. While Miandad's real behavior, feelings and thought process could not be assessed because the behavior, feelings and thought process varies and depends on person to person.

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Keywords: Stress performance, performance anxiety, pressure performance, Javed Miandad, fear and performance, emotions and performance, stress thought process, sports confidence.

1. Introduction:

This is a scientific article addressing the psychological behavior of a person under stressful conditions especially a world renowned celebrity like Javed Miandad (Pakistani cricket player). In order to understand this research article, I recommend that the reader must familiarize himself with the basics of the sports cricket. However, I will provide a little explanation of the sports for the reader who finds it difficult to understand the basics of this sports. Cricket is a baseball like sports started in the 19th century in England (UK).

It is played with a bat and ball with 2 batsmen at the crease at a time unlike baseball, however only one batsman can face the bowling by a bowler at a time. Each team consists of 11 players (Doley, 2009). The match is supervised by 3 umpires, 2 umpires in the ground with the players while the third umpire sits outside the cricket field in the pavilion and helps

umpires make difficult decisions through a TV camera. Cricket is played in three recognized formats at the International Level; the traditional test cricket which is a 5-day match with no bowling overs limit, One Day Cricket which is a 100 over game with each team batting for 50 overs and a newly formed twenty over cricket called as T20.

One Day and T20 are more popular among the cricket fans because the game finishes in less than a day, unlike the 5-day test match. An over is a set of six balls bowled from one end of a cricket pitch. In a single over, a bowler can deliver six balls one after another from one end to the batsman at the other end. The mode and action of bowling are different from baseball (Laws of cricket, 2016). In this article, I will discuss an incident of a historical One-day cricket match played between Pakistan and India.

It is imperative for the reader to understand that in the world of cricket two rivalries are extremely

popular. First, England versus Australia rivalry and second, Pakistan versus India rivalry. Because Pakistan and India have fought 4 wars since the separation, this rivalry supersedes the Australia-England rivalry and most matches are played with immense media hype and pressure (Brett, 2004). Players and fans both take India-Pakistan match very seriously and it becomes nerve wrecking and stressful for the players and the fans to witness a Pakistan-India match. Millions of people watch this encounter between India and Pakistan on TV which makes it far more difficult for the players to perform well and keep their nerves during the match.

On the April 18th, 1986, India and Pakistan played the final of the Australasia Cup in Sharjah where India batted first and gave Pakistan a difficult target of 246 runs to chase in 50 overs. Pakistani players couldn't handle the pressure and lost their batsmen one by one and eventually, only one batsman was left on the crease to face the last ball when 4 runs were needed to win the match. That batsman was Pakistan's legendary batsman Javed Miandad. Javed handled the pressure and hit a 6 on the last ball and lead his team to a nerve wrecking victory (Miandad & Shafqat, 2003).

Javed Miandad's performance was appreciated and discussed all over the world and in fact still is considered the most popular innings of his career (Rizvi, 2014; Aprameya, 2015; Caesar, 2011; Miandad, 2015). Often it was discussed what was in his mind and what was Miandad's feelings before facing the last ball. In this psychoanalytic research article, I will discuss what Javed Miandad's mental and psychological condition was and how he felt before that ball. This article will help us to explore how people behave under immense pressure especially when millions of people are watching us. If we could understand the exact feelings and how those feelings are developed than it would be easy for the people to tackle immense stressful and nerve wrecking conditions like what Miandad faced in that cricket match.

2. Methods:

A comprehensive review of published literature was conducted in PubMed, Embase, MEDLINE, Science Citation Index, JAMA Neurology Journal of Clinical Neuroscience, Journal of Neurology, Neurosurgery, and Psychiatry, Neuropsychiatric Disease and Treatment, Neuropsychology Review, Adaptive Behavior, American Behavioral Scientist, American Journal of Psychology, Annual Review of Psychology, Athletic Insight: The Online Journal of Sport Psychology, Basic and Applied Social Psychology, Behavioral and Brain Sciences, British Journal of Sports Medicine,

British Journal of Psychology, Canadian Journal of Behavioral Science, Canadian Psychology, Health Psychology, The International Journal of Psychoanalysis, Psychological Reports, Bulletin of the Menninger Clinic and various journals of psychiatry and sports medicine.

Some psychiatry journals included British Journal of Psychiatry, Canadian Journal of Psychiatry, Archives of general psychiatry, American Journal of Psychiatry, Journal of Psychiatric research, Psych Info, the Cochrane Library Controlled Trial Registry Databases, some websites and various newspapers. No date restrictions were used. Article relevant to behavior and feelings in stressful situations were searched. Terms for search included but not limited to stressful condition, stressful behavior, fear and performance, anxiety and performance, confidence and performance, confidence and stress, stress in sports, social phobia, pressure and sports performance, Javed Miandad, Miandad last ball six.

I reviewed reference section for additional relevant articles. Article titles and abstracts were reviewed to ascertain if they were applicable to the theme of the stressful condition. Data on stressful and pressured condition appears in a wide range of studies, case series, project descriptions and program evaluations to more formal research trials.

Selected articles were reviewed to identify additional articles that may have been missed by the keyword search. In total, over 500 articles were initially reviewed, with 473 excluded because of little information data on the subject of individual performance in a stressful condition. The study was conducted in the San Francisco Bay Area, California from September 2014 to September 2015 and does not include the involvement of any institution as it was conducted solely by me independently.

Furthermore, I created a questionnaire and collected the answer from 126 people of age group 18 to 59. I made sure all the people I interview are familiar with the sports cricket so that they will be in a better position to understand what Javed Miandad was going through. The survey question was as follows,

Title:

"How people react to pressure and stressful situation?"

The question is,

If you were batting instead of Javed Miandad on the last ball and 4 runs were needed and it was the same match the final India vs Pakistan. You were 110 not out and a batsman of that caliber. What would you be feeling?

A) Fear

B) Anger towards the bowler Sharma

C) Belief and confidence that yes I can hit a sixer

- D) Anger towards your team mates that I wish at least some batsman was playing with me
- E) Nervousness
- F) Happy
- G) Emotionless
- H) Sad - and wish I was not the last man left
- I) Hatred towards your team and the Indian team of putting you in this trouble
- J) Confusion

The results were collected and the data was analyzed and incorporated into the results section of this article.

4. Results:

The results were collected from 126 random people of different age group from 18 to 59 years of age. All the participants in the study were familiar with the sports cricket, Javed Miandad and the incident of the Miandad’s last ball victory and were either Pakistani nationals or Pakistanis living in other countries. Participants were allowed to choose multiple answers. The total of 126 research participants chose 155 responses with some participants choosing more than 1 option. Six participants chose the option “fear” which accounts for 4.8 % of the participants with 3.87 % of the responses out of 155 responses.

Four participants chose anger towards the bowler which is 3.2 percent of the participants and 2.6 % of the total responses. Ninety-three participants chose the option “Belief and confidence that I can hit a six” which is 73.8 % of the participants (60 % of responses). Four participants chose “anger towards your own team mates” which is 3.2 percent of the participants. Thirty-three participants chose nervousness which is 26.2 % of the responses which accounts for 21.2 % of the responses.

Four participants chose the option “happy” (3.2 % participants and 2.6 % responses), 7 chose “emotionless” (5.5 % of participants and 4.5 % of total responses), one person chose “sad feeling” (0.8 % of the participants 0.6 % of the responses), no one chose hatred towards your team and the Indian team while 3 chose confusion 2.3 %, which is 1.9 % of the responses) See Table 1.

Survey Question: If you were batting instead of Javed Miandad on the last ball and 4 runs were needed and it was the same match the final India vs Pakistan. You were 110 not out and a batsman of that caliber. What would you be feeling?

Table 1: shows the questionnaire and the result collected in the survey.

Options	Count
Fear	6 4.8%
Anger towards the bowler Sharma	4 3.2%

Options	Count
Belief And confidence that yes I can hit a sixer	93 73.8%
Anger towards you team mates that I wish at least some batsman was playing with me	4 3.2%
Nervousness	33 26.2%
Happy	4 3.2%
Emotionless	7 5.6%
Sad - and wish i was not the last man left	1 0.8%
Hatred towards your team and the Indian team	0 0%
Confusion	3 2.4%

The results clearly show that most people either are confident when exposed to the high stressed situation as faced by Miandad or perceive Miandad to be confident. The second most popularly selected response was nervousness and the third was emotionless respectively. The results demonstrate that not everyone would react in the same manner. Some people react with confidence (according to their own chosen response) while the others would react with anxiety and nervousness while few claimed that they will be emotionless and some would be happy to face that kind of situation, some sad, angry or confused. Therefore, it could be said that what Javed Miandad was going through totally depends on his personality, not on the situation.

5. Discussion:

This is a scientific study which addresses a question “how people would behave and respond in stressful or pressured situation, especially when millions of people are watching.” What is going on in the mind of a person? These are the scenarios mostly seen by sportsmen or someone who has to perform in front of millions of people like a singer or a stage performer. The stage performer’s mental condition who has to perform in front of a small crowd of few hundred people could be far different than the performer who has to perform in front of millions of people. It is also needed to be studied that if the performance is shown live on TV then will the person feel any different or his feeling would be the same as if he is performing in front of a small crowd.

The subject of this study which is “how people behave in stressful situation” has long been in debate. Whether it is in sports or any other aspect of life. The situation discussed in the introduction and result section is a scenario seen mostly in competitive sports. However, observing this kind of scenario is not very common and is not seen on an everyday basis. Therefore, we could expect that even an experienced sportsman who is habitual of performing in front of a huge crowd in a game which is being broadcasted live,

could feel different if the situation he is exposed to is similar to the one faced by Javed Miandad. For example, in 2004 Euro Cup football (soccer), the world renowned British player David Beckham who is considered as one of the best players in the history of the sports, missed a penalty kick against Portugal and lost the game. As a result, England got knocked out of the Euro Cup. A penalty kick which he would probably never miss in an ordinary match. However, in that match millions of people were watching him and the result of the match was totally dependent on his penalty kick. As a result, Beckham could not endure the pressure and stress and missed an easy penalty (Millward, 2004; McNulty, 2004). The same situation is seen in many other competitive sports where a superstar couldn't endure the stress. This kind of situation is studied in various other sports, for example, De Pero et al studied that the psycho-physiological stress response before and during a competition can affect performance outcome in sports like Team Gym (De Pero et al., 2015). However, some studies showed that physical and psychosocial stress and recovery are key determinants of performance in some other sports (van der Does et al., 2015).

It is still a subject of debate if it depends on the individual sports or it depends on upon the mental make-up of the person facing the situation. For example, Javed Miandad successfully managed to hit a six and lead to a historical victory of his team. However, in the same sports cricket other cricket players who faced the same situation could not hit a six, such as Moin Khan an ex-Pakistani captain under a similar condition in a One Day match in Karachi, Pakistan in March 2004 versus the same opposition India. He could not hit a six despite the match not being a final of any big tournament, as a result, Pakistan lost the game on the last ball (BBC Sports, 2004).

The data collected also point towards this idea that different people would react differently under different conditions. Most people answering, confidence and belief could be due to few reasons. First, that because the research survey question asked "if you were the batsman of that caliber", therefore most people think that to perform at that level you have to be a world-class sportsman of that level and caliber. Moreover, it is also possible that it is their perception of Miandad because Miandad was famous for his fighting spirit. It is also possible that really 73.8 percent people feel confident and this type of scenario brings a motivation and energy which makes people confident.

To study the same phenomena Piacentini et al. studied that endurance competition is a great stressor for psycho-physiology of athletes. They observed that the performance of each athlete is directly correlated

with the cortisol levels in the body. Therefore, it suggests that it is highly likely that Javed Miandad had high levels of cortisol in his body. However, could the same be said about Moin Khan or Beckham? What would be the cortisol level of the person who was under the same situation as Miandad's and was not able to perform well? These are the questions need more research in the future (Piacentini et al., 2015).

6. Confidence and performance:

Javed Miandad and various players playing the same match with him often in TV interviews talked about his confidence (YouTube, 2015). It is said he was confident right from the beginning of the match. The data collected for this research article also suggested that most people either perceived Miandad as confident or most people really are confident under the same or similar situation.

Levy et al conducted a study and demonstrated that that sports confidence had a mediating influence upon the achievement source of confidence-performance relationship. Moreover, cognitive and motivational imagery types were found to be essential sources of confidence, as sports confidence mediated imagery type- performance relationship. They concluded that athletes who construed confidence from their achievements and report multiple images on a more recurrent basis may benefit from augmented levels of state sports confidence and performance (Levy et al., 2014).

It is known that team confidence is beneficial for optimal team functioning and performance. However, little is known about the predictors of team confidence. According to Fransen et al. team enthusiasm was perceived as a most predictive determinant (Fransen et al., 2015). In another study self-confidence has been described as one of the determinants of high performance in sportsmen along with skills (Chidley et al., 2015). This does suggest that it is possible that Miandad really was confident while before the last ball was bowled. However, these kinds of studies point towards another question that whether skill level is associated with the confidence level and ultimately helps to perform well or not. In my opinion, future research will uncover this mystery. In addition, in another study, it is also observed that confidence estimations are effected by opponents' score-related nonverbal behavior (Furley & Schweizer, 2014).

Sports specific theory of confidence was proposed by Vealey in 1986. According to this theory sports confidence could be defined as "the belief or degree of certainty individuals possess about their ability to be successful in sport". This theory measures two factors: trait sports confidence (SC trait) and state sports confidence (SC State). Trait sports confidence



(SC trait) is inherent and considered as a natural character and is relatively stable. Moreover, it relates to a belief of a person about the extent to which their ability brings success in any sports. Therefore, trait confidence is considered global.

On the other hand, state sports confidence (SC State) is a learned behavior and is unstable and changeable. It relates to a person's belief about the extent to which their ability will bring success at one particular moment. Because of this, it is also situation specific.

Vealey (1986) identified a number of strategies to improve state sports confidence (SC State) which are described below.

- Mastery of skill. - When a skill has been developed and the performer perceives progress.
- Styling. Confidence increases if the athlete can demonstrate a highly skilled performance in significant others.
- Physical and mental preparation will increase the chances of successful performance.
- Social reinforcement. Praise and approval by significant others will increase confidence.
- Effective leadership leads to confidence in team members.
- Environmental comfort. People who lack self-confidence will be helped if the working conditions are suitable, for example, the novice should not be observed when learning a new skill.

This model helps in explaining the relationship between general sports confidence and situation-specific sports confidence. Therefore, an athlete who is very successful at one sports transfers much of the confidence derived from his success to other sports situations (Vealey, 1986). This model hints towards an idea that confidence could be present innately or it could be developed. Therefore, the same could be said about Miandad or the people who voted in the study. If he really was confident during that encounter, then the theory of sports confidence could help us understand the reasons for his confidence.

7. Fear, social phobia, and performance:

Around 31 percent (26.2% and 4.8 %) of participants in this study voted that if they were in the same situation as Javed Miandad was, then they would have been nervous or fearful. Why some people answered nervousness and fear are studied in relation to Stage fright or performance anxiety. It is the anxiety, fear, or phobia which may be triggered in an individual when one has to perform in front of an audience. In the context of public speaking, this may precede or accompany participation in any activity involving public self-presentation. Another condition is social anxiety which is a form of anxiety characterized by a fear or discomfort when a person

interacts with others in a social gathering. Usually, the person has a concern of being judged or evaluated by others (Blair et al., 2008).

It is characterized by an immense fear of what others will think about them. It could be referred to as a fear of embarrassment or humiliation, rejection or criticism. This could result in the individual feeling fearful, doubtful, insecure and not good enough (Detweiler et al., 2010). Another kind of anxiety is developmental social anxiety which occurs early in life and is a stage that most children grow out of, but it may persist and grow into chronic social anxiety during their teenage years or in adulthood (National Collaborating Centre for Mental Health, 2013). The chronic social anxiety that causes marked distress and impaired functionality in some parts of daily life are called social anxiety disorder (SAD) (Stein & Stein, 2008) SAD is the most common anxiety disorder, prevalent in 12 % of American adults (Leitenberg, 2013). Common adult forms of social anxiety include performance anxiety, public speaking anxiety, stage fright, and timidity. All of these have a tendency to become anxiety disorders. Clinical and non-clinical forms of social anxiety exist. Both conditions could be differentiated by the intensity and the level of discomfort and the anticipatory nature of fear. The clinical forms are also divided into general social phobia (i.e., social anxiety disorder) and specific social phobia (Stein & Gorman, 2001).

Physical symptoms are commonly seen with social anxiety disorder. These symptoms include blushing, sweating, trembling, palpitations, nausea or stammering. Panic attacks can also occur under intense fear. SAD is considered as an 'illness of lost opportunities' by few authors because many a times sufferers make vital life choices to deal with their illness (Shields, 2004). Participants in this study who chose nervousness and fear or the sportsman who could not endure the pressure situation do they have anxiety or it's a normal feeling in that situation for many people (who do not have any kind of anxiety disorder) is a question which needs to be explored further. However, considering the results produced by Miandad it could be said that it is highly likely that he was not suffering from any anxiety or if he had any kind of anxiety, his confidence overcame that anxious feeling.

Another noticeable thing is the consequences of defensive approach and fear, while Miandad reportedly mentioned by several experts to be confident, the Indian bowler Chetan Sharma in his own interview said he was "so scared". According to him, he didn't want to lose the game for his country (YouTube, 2015). This shows that the fearful feelings with a defensive approach where you try to avoid defeat rather than trying to win leads to a

disappointment. While if you are confident and want to win, most probably you win.

Although 31 percent of research participants claimed that they would be anxious, nervous or fearful under the same circumstances. Javed Miandad according to his fellow players and according to himself was very confident right from the beginning. This showed that to produce good results successfully it is imperative to be confident with a firm belief that "yes I can do it". The discussion and the result section demonstrates that although Miandad who was world renowned sportsman was confident and successfully performed well and lead to a victory of his team due to his confidence and 73.8 % of research participants also voted that they would be confident while being in the same situation, it is also observed that many other world renowned sportsmen when were under the same situation could not perform well and in fact lead to their team's defeat. Some participants in the research claimed to be fearful, anxious and nervous pointing toward social phobia or anxiety. Therefore, it is likely that not everyone would react the same under the similar sort of circumstances.

8. Conclusion:

The behavior under stressful conditions is independent of the situation but is dependent on the individual personality. Some people would react with confidence while the others would react with fear and nervousness under the same situation. However, most people in my survey sample answered that they would react with confidence which could be due to two reasons, either they really are confident in all of their life tasks or it is their perception of Miandad, may it be fearful, confident or fearless. Another limitation of the study could be that the interviewee was shy enough to report their true feelings because if they really are fearful, they are shy to confess that they are actually fearful. Another limitation of the study could be that all the 126 participants in the study were either Pakistani nationals or Pakistanis living in other countries, which could bring the patriotism factor while answering the question.

I believe that this kind of biased in answering the question could affect the data and this research. Moreover, it is also a possibility that most people interviewed knew that to perform at your best under stressful conditions you need to be confident and fearless to produce results like Miandad produced. There are too many possibilities due to the data collected therefore, I believe more research must be conducted on the same topic with a far bigger pool and sample size of participants to get a better idea of how people would react under immense stressful situation especially when millions of people are watching. Why doesn't everybody react the same way

under the same circumstances is a challenging question which I believe would be answered after immense research in the next few decades. Furthermore, Miandad's exact psychological feelings cannot be assessed because it depends on from person to person.

9. Compliance with Ethical Standards:

The author respected compliance with all ethical standards where applicable.

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12. Conflict of Interest:

None.

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