Immersion of lower limbs in water; Effects of selected heavy metals on testosterone secretion in adult male subjects

[1]Okpala C., [2]Ufearo C. S.

[1]Researcher, [2]Professor
Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

1.0 INTRODUCTION

People have contact with body of different types of water for different reasons (Swimming, fishing, off-shore workers, drinking, bathing etc.). Many people that swim as professionals and as occupation (Swimmers, fishermen and other off-shore workers) stay in water for several minutes to hours daily. Water contains Hydrogen and Oxygen in pure state, but in its impure state, water contains many elements which include metals, non-metals, salts and many micro organisms. Most of these waters are in its impure state and contains high concentration of heavy metals (mostly marine water). This may have an effect which may have been ignored based on its insignificant level or based on the understanding that the skin barrier (corneal layer) prevents entry of foreign materials into the body, especially substance with large molecular weight and polarised molecule.[1][2][3] Some researchers have found out that materials like allergens and substance with small molecular weight(less than 1,000 daltons) can pass through the skin.[1][4] Materials can also pass through the skin into the body via intracellular space.[1] Although there are no research works that shows that heavy metals enters the body through the skin. Researchers have shown that barrier function of the skin can be compromised if there is a compromise in the structure of the skin. [4] Other conditions that can cause materials to pass through the corneal layer includes; high concentration of penetrating substance, if the substance is non polar or semi polarised but small in molecular size {<500 daltons}.[3] There are no literature on the effects of heavy metals in the body in relation to male reproductive hormone secretion.

Greater population of those living in riverine area in southern Nigeria are offshore workers (including fishermen and marine transporters). This set of people is involved in swimming either directly or indirectly, some engage themselves in swimming as a hobby while others like the professional swimmers do it in order to earn a living[5]. They immerse their lower limb in the water (marine or fresh) as they do their job every day. As a result of this, they come in contact with water body on daily basis. There are so many literatures on effects of swimming on other systems of the body of mammals but little in humans. There is little or no research done on the effect of interaction between heavy metals and the body during swimming on endocrine and reproductive system especially on testosterone hormone. Testosterone is very important in the life of all male mammals following the central role it plays in fertility and development of secondary sexual characters in male mammals. There is need to have a research proof or evidence-based research on the effects of selected heavy metals on testosterone secretion in adult male subjects during immersion of lower limb in water.

2.0 MATERIAL AND METHODS

Thirty-three healthy young adult males that participated in this study were randomly selected based on acceptance, and they were recruited from Nnamdi Azikiwe University, Nnewi campus over a period of one month. Ethical clearance was obtained from the ethics and research committee of Nnamdi Azikiwe University, Faculty of Basic Medical Sciences, Nnewi Campus. Those who were aged 18 and above, that has not been diagnosed of diabetes, chronic obstructive pulmonary disease, pituitary tumor, sleep apnea or takes opoid, glucocorticiod drugs and does not take dopamine receptor stimulatory or inhibitory drugs were recruited in this research. Oral consent of the participant was obtained. The selected volunteer formed the study groups and a questionnaire was given to them to fill in other to collect their bio-data. A scale balance and a stadiometer were used to obtain their weight and height. The BMI of each participant was calculated using this formula according to Kolimechkov, 2016 and Centers for Disease Control and Prevention (CDC), 2009.

BMI = (weight in kilograms) divided by (height in meters X height in meters)

BMI= Weight (kg)/Height (m^2) [6][7]

Marine water was fetched from the Atlantic Ocean in Abonema, Rivers State and Fresh water was also fetched from "Nwosu" stream in Akpo Aguata Local Government Area Anambra State. Distilled water was bought from bridge-head market Onitsha a special market for medications and medical equipment in Anambra State. Fifteen litres buckets were rented, divided into three groups and labelled differently from other groups based on their water content. Buckets labelled "A" contained marine water obtained from Atlantic Ocean, buckets labelled "B" contained Fresh water obtained from Nwosu stream while those labelled "C" contained distilled water. The procedure followed by Okpala *et al*, was adopted in this research, the participants were divided into three groups based on the source of the water. First group which their buckets were labelled 'A' had marine water, and second group which there bucket were labelled 'B' had fresh water and the third group which there bucket were labelled 'C' had distilled water.

The participants were educated about the process of the experiment and the participants were asked to wash their legs with tap water before they immerse their legs into the water up to the kneel level according to their groups for one hour everyday for fifteen days, blood sample for serum testosterone analysis were taken from each participant twice (before immersion and after immersion) on intervals as follows: 1st day, 5th day, 10th day and 15th day. The samples were taken in the morning between 8.00am and 10am

intravenously from their arm using syringe [5]. Blood sample collected was introduced into a plain tube. The blood samples were centrifuged at 2,500 rpm, serum separated, stored at -80ºC in freezer, analysed in a single session to reduce interassay variation and by a lab technician.
The parameter used to determine testosterone hormone secretion in the subjects was the concentration of testosterone hormone in the blood serum. The serum levels of testosterone were assayed using ELISA technique.
Data obtained was statistically analysed using Students sample t-test method [SPSS version 21.0]. Data was expressed as mean ± SEM *p<0.05

3.0 RESULTS

The mean Age and BMI of the subjects are shown in Figure 1 and Figure 2 respectively. There were no significant difference between ages in group A (marine water) vs group C/control (distilled water) (20.70±0.50yrs vs 20.55±0.59yrs; p-value 0.769) and group B (fresh water) and group C/control (distilled water)(21.00±0.55yrs vs 20.55±0.59yrs; p-value 0.817). BMI of the subjects in group B (fresh water) (22.47±0.40 kg/m) shows a significant difference when compared with group C/control (distilled water)(21.43±0.72 kg/m) p-value = **0.024*** while there were no significant difference between group A (marine water)(23.11±0.97kg/m) and group C/control (distilled water)(21.43±0.72 kg/m) p-value = 0.120.
The plasma testosterone response to lower limb immersion in marine water at variable time is shown in Figure 3 a negative response was observed from the result throughout the period of the exposure. There was a significant difference between the mean difference of Day-0 vs Day-5(-1.17 ± 0.43 vs 0.02 ± 0.51; p-value **0.043***) but Day-10 (-1.00 ± 0.32) and Day-15 (-0.46 ± 0.35) are higher than Day-0 (-1.17 ± 0.43) but not statistically significant p-value = 0.613 and 0.070 respectively.
The plasma testosterone response to lower limb immersion in fresh water at variable time is shown in Figure 4. The result shows a negative response on Day-0, Day-10 and Day-15 while Day-5 shows a positive response during the time of exposure. When the mean difference of Day-0 (-0.32 ± 0.39) is compared with others, it shows that day-5 (0.60 ± 0.41) is statistically higher than Day-0 (-0.32 ± 0.39) p-value = **0.046*** but Day-10 (-0.05 ± 0.18) and Day-15 (-0.23 ± 0.18) are higher but not statistically significant p-value = 0.172 and 0.634 respectively.
The plasma testosterone response to lower limb immersion in distilled water at variable time is shown in Figure 5. A positive response was observed on Day-0 (0.24 ± 0.36) and Day-10 (0.17 ± 0.28) while Day-5 (-0.16 ± 0.36) and Day-15 (-0.19 ± 0.16) shows a negative response during the time of exposure. When the mean difference of Day-0 (0.24 ± 0.36) is compared with others, it shows that day-15 (-0.19 ± 0.16) is statistically lower than Day-0 (0.24 ± 0.36) p-value = **0.020*** but Day-5 (-0.16 ± 0.36) and Day-10 (0.17 ± 0.28) are lower but not statistically significant, p-value = 0.293 and 0.813 respectively.
The Plasma testosterone response to lower limb immersion in different water sample on Day-0 is shown in Figure 6. Result shows that those exposed to marine water (-1.17 ± 0.43) had a statistically lower/negative response than those exposed to distilled water (control group) (0.24 ± 0.36) p-value = **0.009*** while those exposed to fresh water (-0.32 ± 0.39) were lower than those exposed to distilled water (0.24 ± 0.36) but not statistically significant, p-value = 0.183.
The Plasma testosterone response to lower limb immersion in different water sample on Day-5 is shown in Figure 7. Observation shows that those exposed to marine water (0.02 ± 0.51) and fresh water (0.60 ± 0.41) were higher than those exposed to distilled water (control group) (-0.16 ± 0.36) but not statistically significant, p-value = 0.731 and 0.091 respectively.
The Plasma testosterone response to lower limb immersion in different water sample on Day-10 is shown in Figure 8. Result shows that those exposed to marine water (-1.00 ± 0.32) had a statistically lower/negative response than those exposed to distilled water (control group) p-value= **0.006*** while those exposed to fresh water (-0.05 ± 0.18) were lower than those exposed to distilled water (0.17 ± 0.28) but not statistically significant, p-value = 0.259.
The Plasma testosterone response to lower limb immersion in different water sample on Day-15 is shown in Figure 9. Observation shows that those exposed to marine water (-0.46 ± 0.35) and fresh water (-0.23 ± 0.18) were lower than those exposed to distilled water (control group) (-0.19 ± 0.16) but not statistically significant, p-value = 0.455 and 0.811 respectively.

Table 1: Concentration of selected heavy metals present in different water samples

S/N	Parameters	Marine water	Fresh water	Distilled water	WHO standard
1	Arsenic (ppm)	0.025	0.037	0.017	0.01*
2	Chromium (ppm)	0.00	0.00	0.00	0.05*
3	Mercury (ppm)	1.200	0.300	0.500	0.01*
4	Cadmium (ppm)	0.422	0.000	0.333	0.10*
5	Lead (ppm)	0.20	0.13	0.17	0.10*

- - citation for WHO standard [8][9][10]

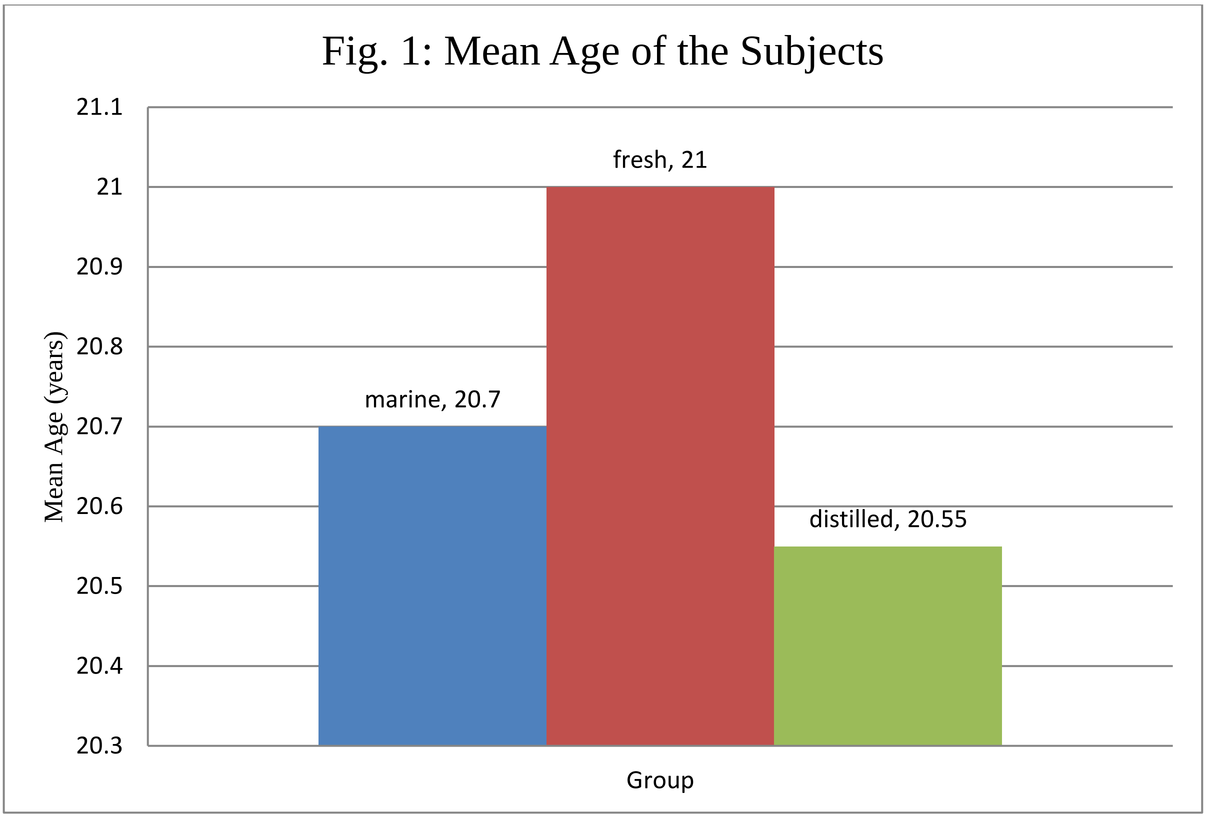


Fig. 1: Mean Age of the Subjects
21.1
21
20.9
20.8
20.7
20.6
20.5
20.4
20.3
Mean Age (years)
fresh, 21
marine, 20.7
distilled, 20.55
Group

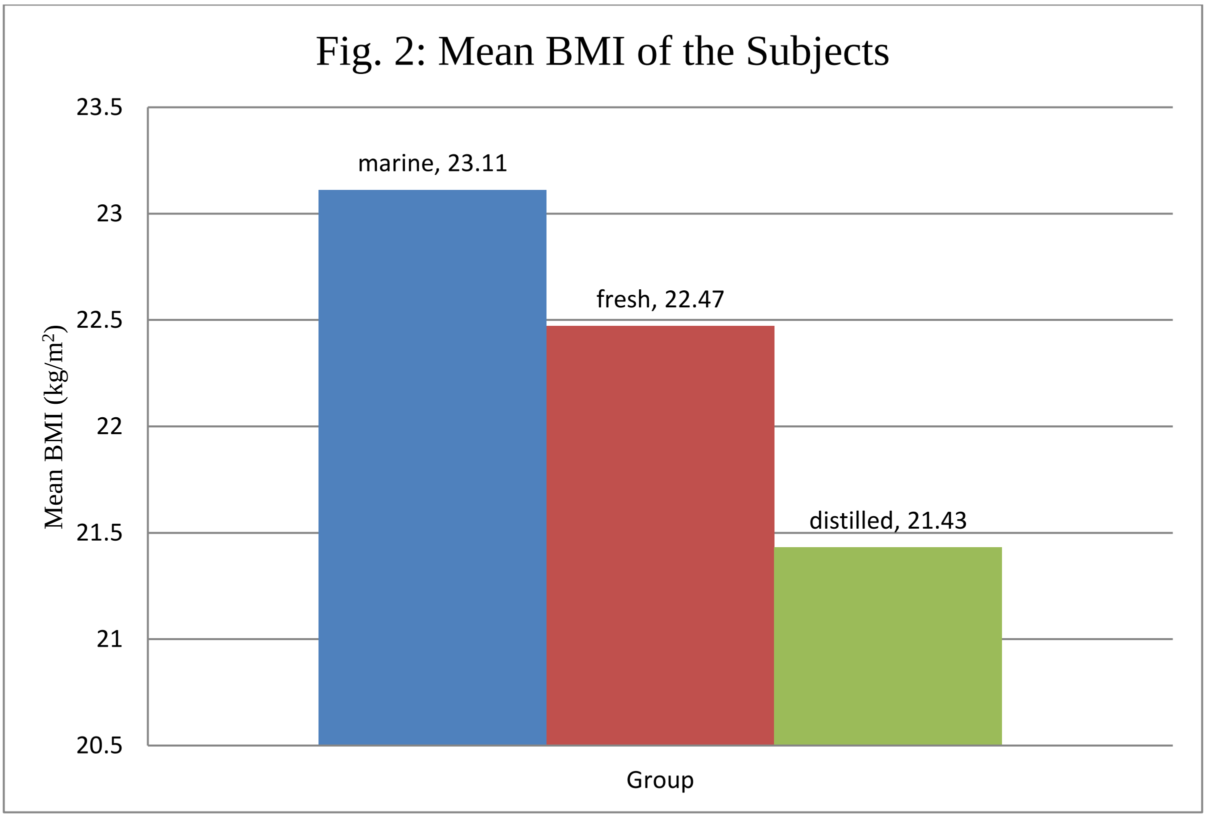


Fig. 2: Mean BMI of the Subjects
23.5
23
22.5
22
21.5
21
20.5
Mean BMI (kg/m2)
marine, 23.11
fresh, 22.47
distilled, 21.43
Group

Fig. 3: Plasma testosterone response to lower limb immersion in marine water at variable time interval.

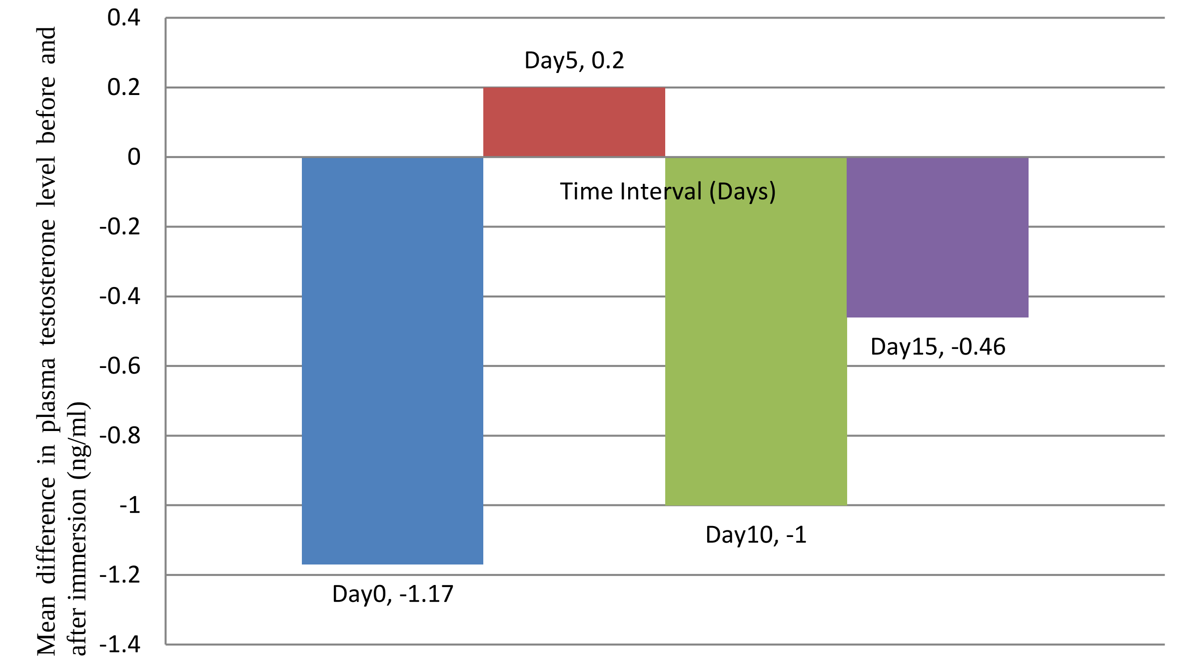
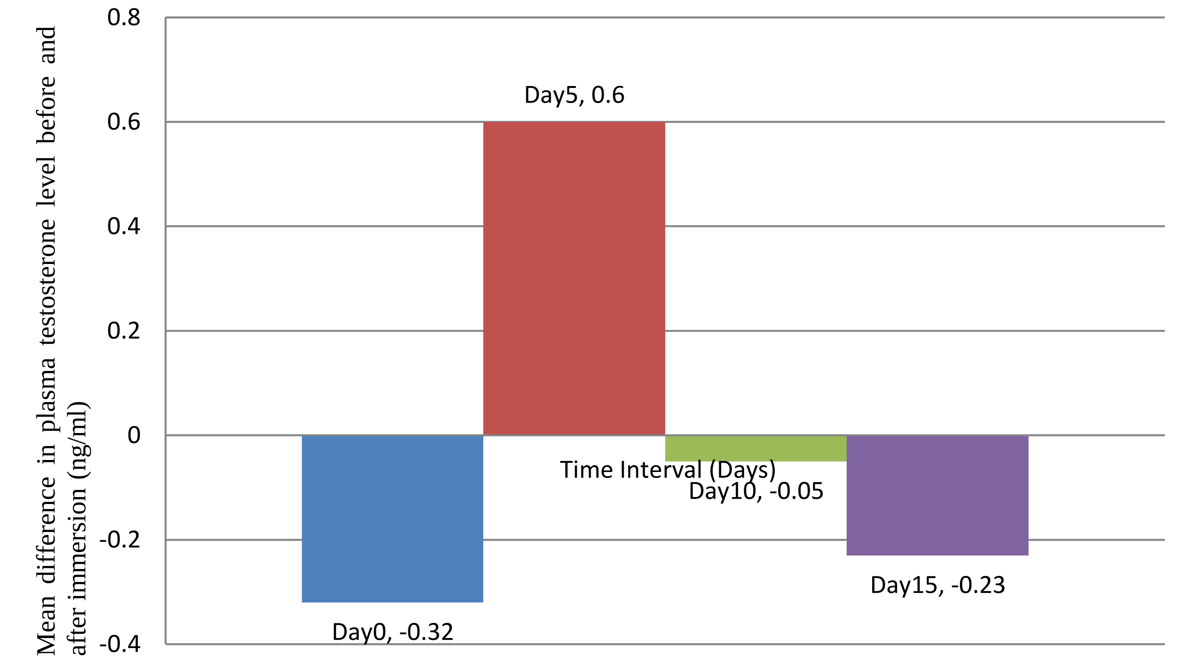


Fig. 4: Plasma testosterone response to lower limb immersion in fresh water at variable time interval.



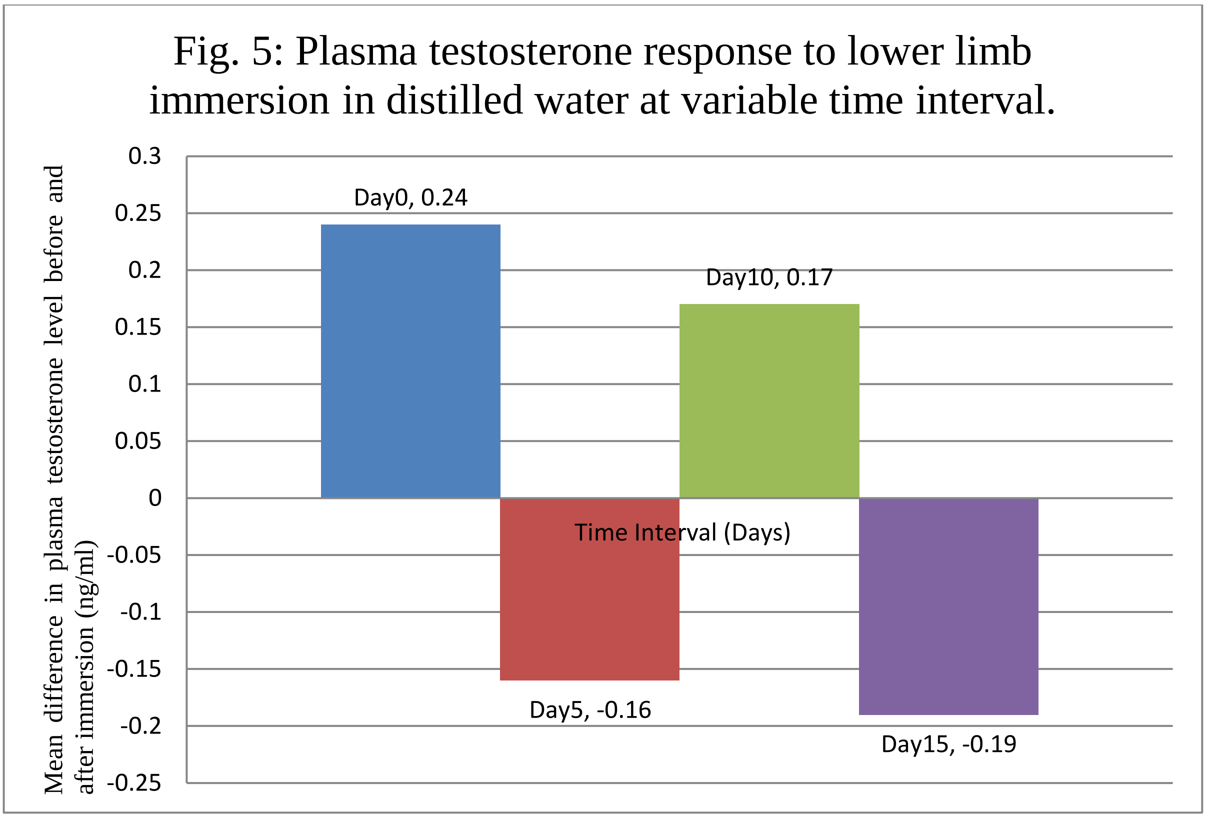


Fig. 5: Plasma testosterone response to lower limb immersion in distilled water at variable time interval.
Mean difference in plasma testosterone level before and after immersion (ng/ml)
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
Day0, 0.24
Day10, 0.17
Time Interval (Days)
Day5, -0.16
Day15, -0.19

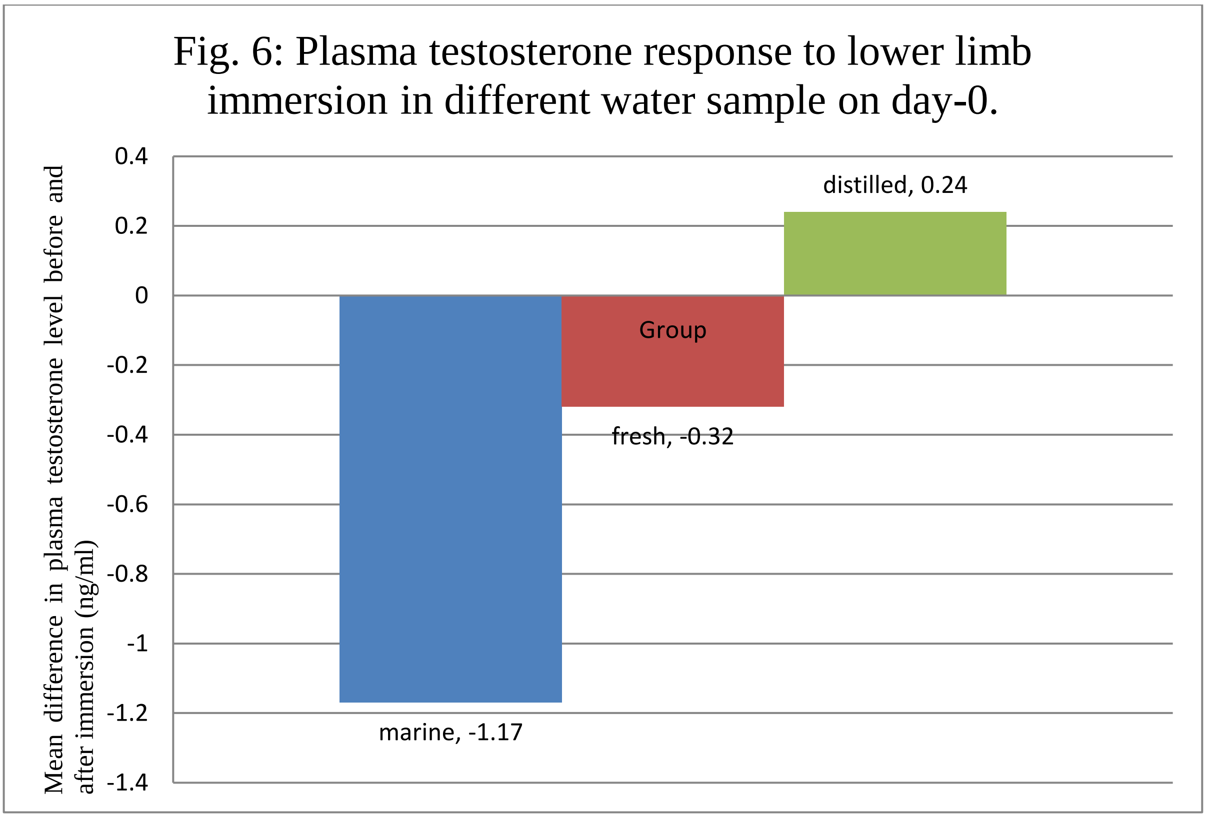


Fig. 6: Plasma testosterone response to lower limb immersion in different water sample on day-0.
Mean difference in plasma testosterone level before and after immersion (ng/ml)
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
-1.4
distilled, 0.24
Group
fresh, -0.32
marine, -1.17

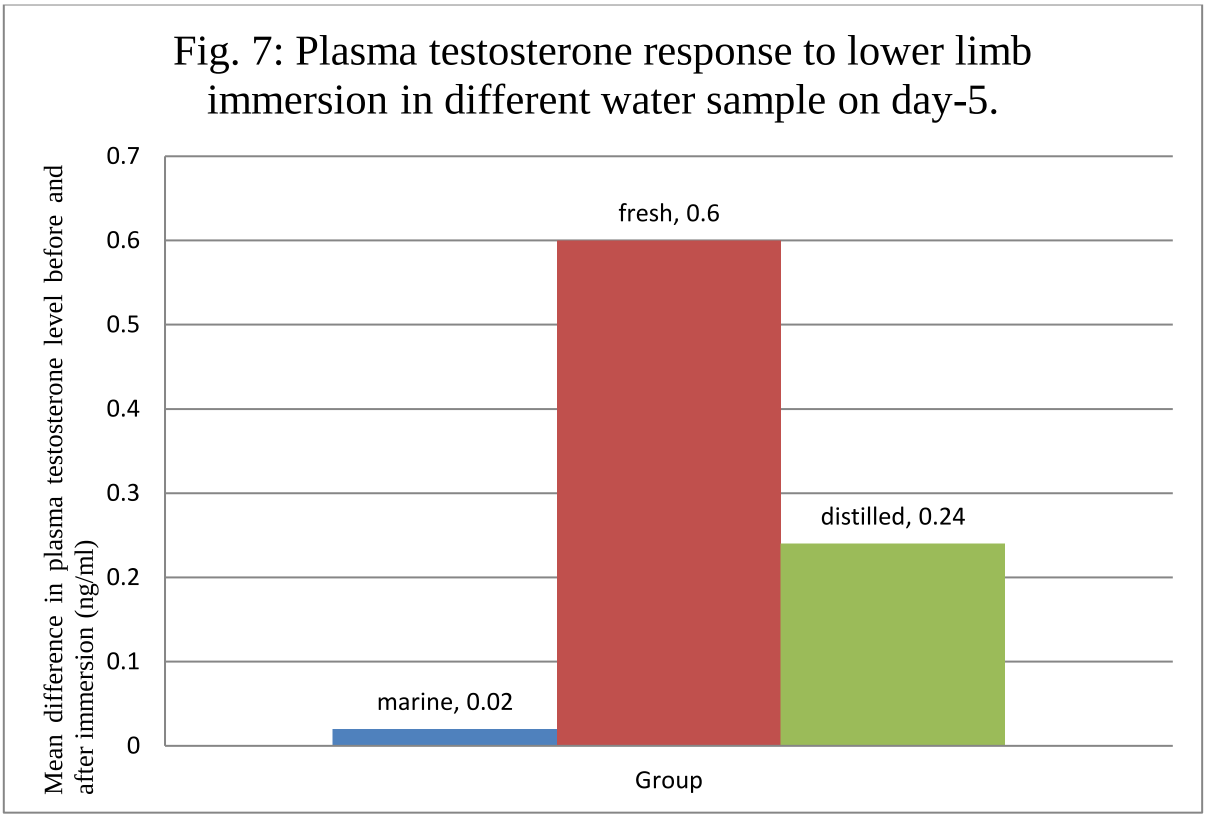


Fig. 7: Plasma testosterone response to lower limb immersion in different water sample on day-5.
Mean difference in plasma testosterone level before and after immersion (ng/ml)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
fresh, 0.6
distilled, 0.24
marine, 0.02
Group

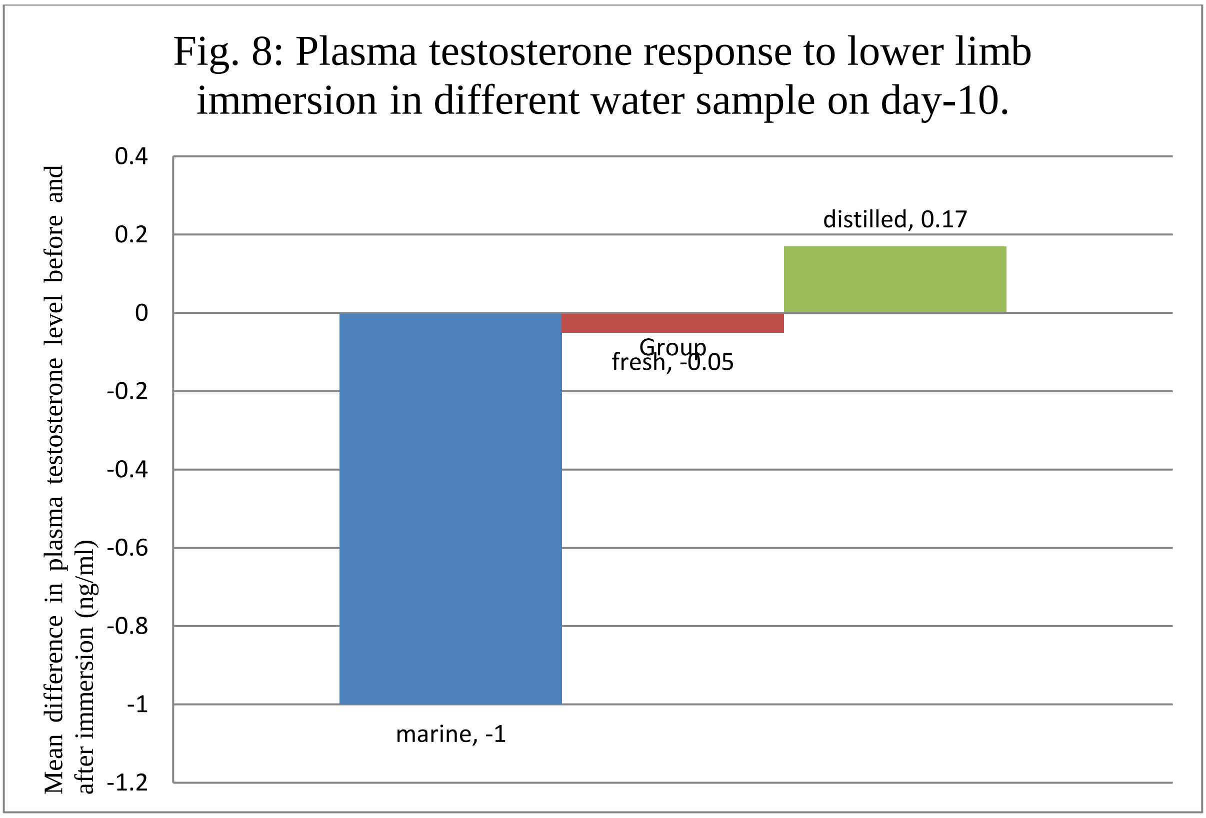
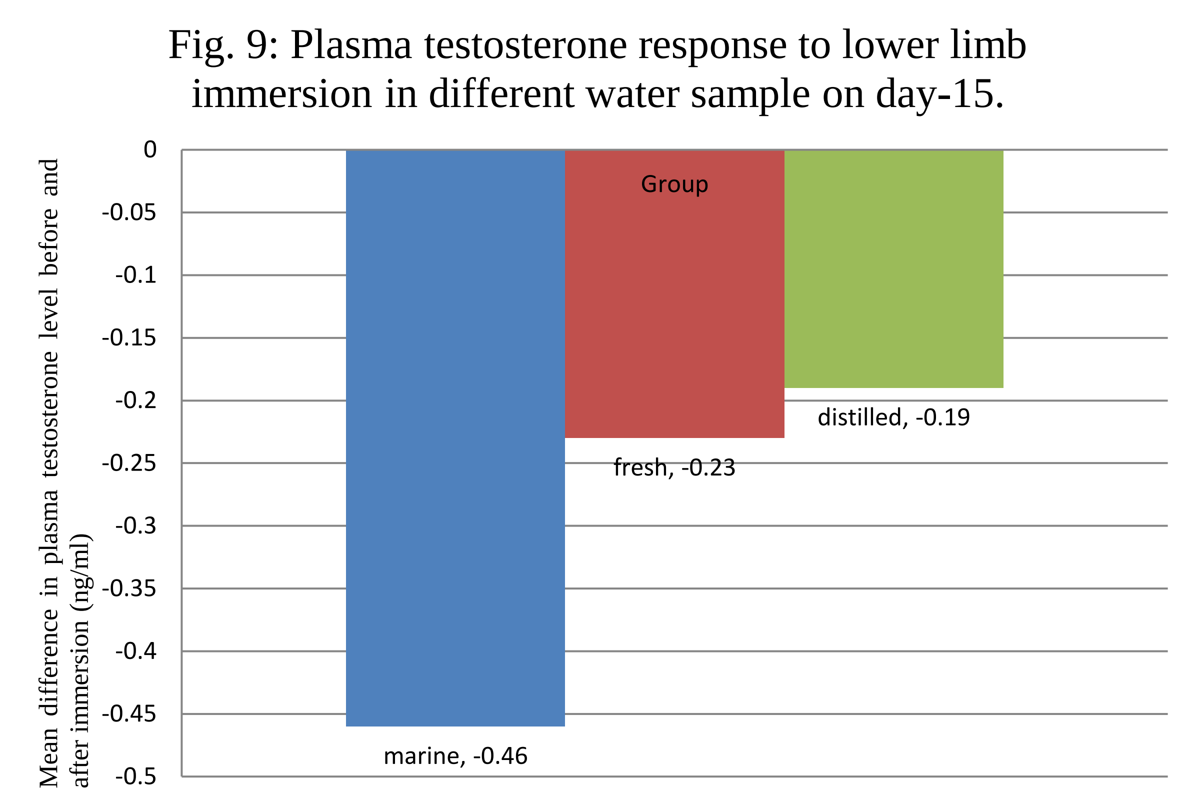


Fig. 8: Plasma testosterone response to lower limb immersion in different water sample on day-10.
Mean difference in plasma testosterone level before and after immersion (ng/ml)
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
-1.2
distilled, 0.17
Group
fresh, -0.05
marine, -1



4.0 DISCUSSION

Normal BMI (BMI < 25kg/m^2) subjects were recruited to avoid incidence of low testosterone due to obesity.[11] Corona *et al*, has both reported in their different research that higher BMI leads to low serum testosterone.[12] Shamim *et al*,; Osuna *et al*, has both reported a negative correlation between testosterone and BMI in their various studies.[13][14]

The median and modal ages for all groups however lie between 19 and 21 years. The younger age used in this research help to eradicate the possibilities of low testosterone due to old age. Age above 50years has been reported to correlate with decrease serum testosterone.[15]

The negative response to testosterone secretion by the subjects on Day-0, Day-10 and Day-15, as shown in (Fig. 3) may be as a result of the high concentration of heavy metals present in the marine water.

High concentration of some heavy metals like cadmium, arsenic, mercury and lead in marine water suggest that they contributed in negative response observed among the subjects exposed to marine water. Absorption through contact with the skin can be possible for some heavy metals like Cadmium [16], Arsenic, Mercury and Lead [17]. Several researches reported testosterone suppression among occupationally Lead exposed men. Suppression of testicular testosterone levels and increasing steroid binding globulin levels related to increased duration of exposure to Lead has been also demonstrated among mice exposed to Lead for 30 days[18][19][20], Another researcher (Heath *et al*), reported a decrease in testicular and plasma testosterone among animals exposed to Mercury[21]. Barregard *et al*, reported a decrease in the serum testosterone among men exposed to inorganic Mercury [22]. Webb *et al*, reported a negative correlations between serum testosterone and Mercury content, they also suggested the ability of the Mercury to disrupt endogenous hormone levels in yellow-fin sea-bream [23]. Aliakbar *et al*, observed that environmental pollutants mimic the sex hormone by binding to androgen receptors (AR) and influencing cell signalling pathways [24]. Studies on animals show that Cadmium causes a decrease in testicle weight, in the amount of testosterone produced, and in sperm count and activity. A reduced sperm count is caused by disturbances in the cell cycle, DNA repair and cell proliferation [25][26]. Ranjit *et al*, reported a decrease in serum testosterone among mice exposed to Arsenic [27]. This work is consistent with previous reports where the serum testosterone level showed a negative response to exposure to heavy metals.

Day-5 shows a positive response to testosterone secretion, this may be as a result of the body trying to augment for the deficit recorded previously as a result of the exposure by freeing more protein bound testosterone in the blood. When result obtained from Day-5, Day-10 and Day-15 is compared with Day-0 as shown in (Figure 3), it shows a significantly higher plasma testosterone concentration in Day-5 than in Day-0. This may likely be as a result of the body trying to re-adjust to the new environment.

The results for those exposed to fresh water were similar to those exposed to marine water but shows less negative response to testosterone secretion by the subjects as shown in (Figure 4). This may be as a result of concentration difference of individual substance present in the two water samples. Fresh water contains less concentration of heavy metals except for Arsenic which is higher in concentration in fresh water than other water samples used in this research. This reduced concentration of heavy metals in fresh water may be responsible for the positive response observed in those exposed to fresh water than in other water samples. Studies have shown that there is a decrease in testicular and plasma testosterone among animals exposed to mercury [21]. Two researchers separately reported a decrease and negative correlation in serum testosterone in men exposed to inorganic mercury [22][23]. Absence of Cadmium may largely be responsible for positive response to testosterone secretion among those exposed to

fresh water. Aliakbar *et al*, observed that environmental pollutants mimic the sex hormone by binding to androgen receptors (AR) and influencing cell signalling pathways [24]. Studies on animals show that Cadmium causes a decrease in testicle weight, in the amount of testosterone produced, and in sperm count and activity. A reduced sperm count is caused by disturbances in the cell cycle, DNA repair and cell proliferation [25][26]. This research supports the findings of previous reports on the effects of some heavy metals especially Cadmium on serum testosterone level.
Those exposed to distilled water shows a fluctuating pattern in serum testosterone as was observed in (Figure 5), this is opposite of what was obtained in test group which may be attributed to the nature of water (distilled water). The heavy metals present in the distilled water sample were not too high or low to cause a constant pattern in testosterone secretion/concentration. Normal pulsative pattern of testosterone secretion may be responsible for the fluctuations noticed in subjects in control group.
The result of this research shows that the plasma testosterone level in those exposed to marine water were significantly lower on Day-0 and Day-10 when compared with result of those exposed to distilled water (control group) while the plasma testosterone level of those exposed to marine water on Day-5 and Day-15 were lower than those exposed to distilled water (control group) as shown in (Fig. 6, Fig. 7, Fig. 8 and Fig. 9) but not statistically significant. This maybe as a result of concentration difference in heavy metals present in the different water samples.
The results for those exposed to fresh water when compared with those exposed to distilled water (control group), shows no significant difference in plasma testosterone level.
There were scarce literatures on this area, from the results of this research it could be suggested that exposure to some heavy metals especially Cadmium, Mercury, Lead And Arsenic through immersion of lower limb in water has a negative effect on testosterone secretion in adult male but the mechanism of action for the depressive activities is not well understood.

5.0 CONCLUSION

After considering the results of the different groups, there was indication that plasma testosterone level was lower in those exposed to marine water than in fresh water and distilled water (control group) this suggest that high concentration of heavy metals in marine water may be responsible for the depressive action on testosterone secretion but physiological adaptation may tend to restore the effects with time.

RECOMMENDATION

Further research should be conducted in this area on long term duration of about six months and above to understand the effects better.

REFERENCES

1. Christofori M. Nastiti, Thellie Ponto, Eman A., Jeffrey E. Grice, Heather A. E. Benson, Michael S. Robert: Topical Nano and Microemulsions for Skin Delivery. Pharmaceutics (2017) 9:37; doi: 10.3390/pharmaceutics9040037
2. Prausnitz R. Mark, Elias M. Peters, Franz J. Thomas, Schmuth Matthias, Jui-Chen Tsai, Menon K. Gopinathan, Holleran M. Walter, Feingold R. Kenneth: Skin Barrier and Transdermal Drug Delivery, Textbook of MEDICAL THERAPY (2012).
3. Leveque J, Ribaud C, Garson J.: Caractérisation biophysique du stratum corneum. Relation structure-fonctions. Pathol Biol (1992); 40: 95-108.
4. Frederic Berard, Jean-Paul Marty and Jean-Francis Nicolas.: Allergen penetration through the skin. European Journal of Dermatol (2003); 13:324-30.
5. Okpala Chukwuebuka and Ufearo C. S. Effect of immersion of lower limb in marine water on testosterone secretion in adult male subjects. Journal of Medical Science and Clinical Research (2022) Vol. 10, Issue 04, page 81-91.
6. Kolimechkov, Stefan: Body Mass Index. www.stk-sport.co.uk. (2016) DO - 10.13140/RG.2.2.31492.94086
7. Centers for Disease Control and Prevention [CDC],: About BMI for children and teens. (2009) Available at: http://www.cdc.gov/healthyweight/assessing/bmi/childrens_bmi/about_childrens_bmi.html
8. Morais Simone, Fernando Garcia e Costa and Maria de Lourdes Pereira: Heavy Metals and Human Health. Environmental Health – Emerging Issues and Practice (2012)
9. United States Environmental Protection Agency (USEPA).: Arsenic in drinking water fact sheet. 10.07.2011, (2005a) Available from http://www.epa.gov/safewater/ arsenic.html
10. World Health Organisation (WHO).: Guidelines for drinking-water quality. Sixty-first meeting, Rome, (2004a) 10-19. June 2003. Joint FAO/WHO Expert Committee on Food Additives
11. Eriksson Joel, Haring Robin, Niels Grarup, Liesbeth Vandenput, Henri Wallaschofski, Erik Lorentzen, Torben Hansen, Dan Mellström, Oluf Pedersen, Matthias Nauck, Mattias Lorentzon, Lise Lotte Nystrup Husemoen, Henry Völzke, Magnus Karlsson, Sebastian E. Baumeister, Allan Linneberg and Claes Ohlsson: Causal relationship between obesity and serum testosterone status in men: A bi-directional mendelian randomization analysis. PMCID: PMC5407807. PLoS One. (2017) 12(4): e0176277.
12. Corona G, Rastrelli G, Monami M, Saad F, Luconi M, Lucchese M,;. Body weight loss reverts obesity-associated hypogonadotropic hypogonadism: a systematic review and meta-analysis. European journal of endocrinology / European Federation of Endocrine Societies. (2013) 168(6):829–43. [PubMed]
13. Shamim M.O, Khan F.M.A, Arshad R.: Association between serum total testosterone and Body Mass Index in middle aged healthy men. Pak J Med Sci; (2015) 31(2) 1-6.

14. Osuna JA, Perez G, Bellabarba A, Villaroel V.: Relationship between BMI, total testosterone, sex hormone binding-globulin, leptin, insulin and insulin resistance in obese men. Arch Androl. (2006) 52(5):355-361
15. Le B., Chen H., Zirkin B., Burnett A.: New targets for increasing endogenous testosterone production: clinical implications and review of the literature. Andrology, (2014) 2, 484-490. American Society of Andrology and European Academy of Andrology.
16. Alaee S, Talaiekhozani A, Rezaei S, Alaee K, Yousefian E. : Cadmium and male infertility. Journal of infertility and Reproductive Biology. (2014) Volume 2, Issue 2, Pages 62-69.
17. Al Osman Muwaffak . Yang Fei . Massey Isaac Yaw: Exposure routes and health effects of heavy metals on children. Springer Nature B.V. (2019)
18. Mohsen Vigeh, Derek R. Smith, Ping-Chi Hsu: Review article; How does lead induce male infertility? Iranian Journal of Reproductive Medicine (2011) Vol.9. No.1. pp:1-8
19. Alexander BH, Checkoway H, Faustman EM, van Netten C, Muller CH, Ewers TG: Contrasting associations of blood and semen lead concentrations with semen quality among lead smelter workers. (1998) Am J Ind Med; 34: 464-469.
20. Rodamilans M, Osaba MJ, To-Figueras J, Rivera Fillat F, Marques JM, Perez P: Lead toxicity on endocrine testicular function in an occupationally exposed population. Hum Toxicol; (1988) 7: 125-128.
21. Heath J.C, Abdelmageed Y, Braden T.D, Goyal H.O: The Effects of Chronic Ingestion of Mercuric Chloride on Fertility and Testosterone Levels in Male Sprague Dawley Rats. J Biomed Biotechnol. (2012) 815186.
22. Barregard L, Lindstedt G, Schutz A, Sallsten G: Endocrine function in mercury exposed chloralkali workers. *Occupational and Environmental Medicine*. (1994) 51(8):536–540.
23. Webb, M.A.H., Feist G.W., Fitzpatrick M.S., Foster E.P., Schreck C.B., Plumlee M., Wong C. and Gundersen D.T.: Mercury Concentrations in Gonad, Liver and Muscle of White Sturgeon Acipenser transmontanus in the Lower Columbia River. Archive of Environmental Contaminant and Toxicol., (2006) 50: 443-451
24. Aliakbar Hedayati, Parviz Zare and Safoura Abarghouei: Effects of Environmental Mercury on Some Hormonal Parameters of the Main Mariculture Fish of Persian Gulf. Global Veterinaria (2012) 8 (1): 43-50
25. Ren X.M., Wang G.G., Xu D.Q., Luo K., Liu Y.X., Zhong Y.H: The protection of selenium on cadmium-induced inhibition of spermatogenesis via activating testosterone synthesis in mice. Food ChemToxicol. (2012), 50(10): 3521-3529. DOI: 10.1016/j.fct.2012.07.021
26. Yari A., Asadi M. H., Bahadoran H., Dashtnavard H., Imani H., Naghii M.R.: Cadmium toxicity in spermatogenesis and protective effects of L-carnitine in adult male rats. Biol. Trace Elem. Res. (2010), 137: 216-225. DOI: 10.1007/s12011-009-8577-5
27. Ranjit Kumar, Shabbir Ali Khan, Pushpalata Dubey, Nath A., Singh J.K., Ali M.D.
and Arun Kumar: Effect of arsenic exposure on testosterone level and spermatogonia of mice. World Journal of Pharmaceutical Research (2013) Volume 2, Issue 5, 1524-1533.