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Application of some medicinal Plants to eliminate *Trichodina* sp. in tilapia (*Oreochromis niloticus*)

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Abstract: Medicinal plants are important elements of traditional medicine in virtually all cultures and product promise a cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for all problems which groupers culture faces today. The control of Trichodinaiasis and Aeromonus hydrophila in ponds of stocked tilapia with any antiprotozoal and antibacterial agent at present is evidently a cost. In addition, possibly leaves toxic residues in tilapia and mortality. Also, affect for a short times. For the previous reasons, the medicinal plants instead. Garlic (Allium sativum) and Artemisia vulgaris as optional medicinal plants to treat fish Trichodina sp and Aeromonas hydrophila . 350 Tilapia *O.niloticus* (average weight 100 ±20 g) derived from a private fish farm in Kafr El Sheikh governorate infested with Trichodina sp, were kept in cement ponds (3×8 metre) and sex diets were formulated to contain different levels of Allium sativum extract (1, 4 and 8g/kg diet) and Artemisia vulgaris extract (1, 3, and 4.5g/kg diet) added. The results showed that crude extracts of either garlic or Artemisia vulgaris at 800 mg/l significantly ($P < 0.05$) eliminated Trichodina sp and Aeromonas hydrophila infections in tilapia. garlic and Artemisia vulgaris will be used as an alternatives to chemicals to treat of infected tilapia with *Trichodina and Aeromonus* sp. [Report and Opinion, 2009;1(6):1-5]. (ISSN 1545-4570).

Key words: Trichodina sp, Aeromonas hydrophila , tilapia, medicinal plant, garlicArtemisia vulgaris

1. Introduction:

Tilapia (*Oreochromis niloticus*) is one of many economical freshwater fish that are cultured worldwide and the third most commonly farmed fish after carp and salmon with global production of 1.49 million metric tonnes (mmt) in 2002, and Farmed tilapia is exceeded two million metric tons in 2004 worldwide (Fitzimmons, 2003). Today plant materials are present in, or have provided the models for 50% of western drugs (Robbers et al., 1996). At first, tilapia were considered to be more resistant to bacteria and parasitic diseases than other species of cultured fish. However, in more recent times, tilapias have been found to be most susceptible to both bacterial and parasitic diseases (Philip et al., 2008).Common tilapia pathogens are Aeromonas hydrophila and Trichodina sp. Aeromonas and Trichodina sp in tilapia has become an increasing problem and are leading to diseases that cause severe economical impact. Chemotherapy is widely used to control infectious bacterial and parasitic diseases. The use of chemicals in treating health problems has also been complicated by the misleading advice provided to the farmers by feed and chemical companies regarding the use of antibiotics and other therapeutic drugs. It has been used in developing countries as well as using extended to developed countries (Lanfranco, 1999). Plant extracts decrease the selective pressure for developing antibiotic resistance (Lewis & Ausubel, 2006). The screening of plant extracts and natural products for antimicrobial activity has shown that higher plants represented a potential source of new anti-infective agents (Press, 1996). Controlling of Trichodinaiasis with freshly prepared potassium permanganate (Eissa, 2002), but it now very expensive and of no effect in the presence of organic matter. In correspond, there is a fast growing interest in screening antiparasitic and antibacterial substances from plants to replace antiparasitic and disinfectant alternatives. Two such plants are garlic and Artemisia vulgaris. Garlic is one of the edible plants that had a strong interest to scientists and recognized as an important medicinal plant which has a wide spectrum of actions; not only antibacterial and antiprotozoal, but also has beneficial effects on the immune systems (Harris et al., 2001). In addition to their effects, activities against the variety of Gram-negative and Gram-positive were and continue to be extensively investigated (Whitemore & Naidu, 2000). A wide range of microorganisms including bacteria and protozoa have been shown to be sensitive to crushed garlic preparations and can help in the control of pathogens, especially bacteria, and increase the welfare of fish (Corzo-Martinez et al, 2007;). Madsen et al. (2000b) reported that raw and squeezed garlic (Allium sativum) at 200 mg/l had Report and Opinion 2009 potential to treat Trichodinaiasis in eel. Artemisia vulgaris is another plant that is promising to prevent fish diseases (Shagnliang et al, 1990) and (Direkbusarakom, 2004), thus their attention to plants for medicinal use.

Using the crude extract from either garlic or Artemisia vulgaris are one of the new challenging methods for Trichodinaiosis treatment.The aim of this present research was to (1) determine the efficacy of garlic and Artemisia vulgaris as cheap antibacterial medicinal plants alternatives to control Trichodinaiosis epizootic in tilapia .

2- Materials and Methods

2-1-Fish

Tilapia *O.niloticus* (average weight 100 ±20 g) derived from a private fish farm in Kafr El Sheikh governorate infested with Trichodina sp , were kept in cement ponds (3×8 metre) and supplied with well-aerated freshwater using compressed air. Cement ponds were daily cleaned, and the water exchange rate per day, including fish feces and remaining food, was approximately 25% of the total volume. The water temperature was adjusted (26-27°C) by a thermostat column heater in each pond, with high density for 20 days.

2-1-Diets

Sex diets were formulated to contain different levels of Allium sativum extract (1, 4 and 8g/kg diet) and Artemisia vulgaris extract (1, 3, and 4.5g/kg diet). Control diet was free from both Allium sativum and Artemisia vulgaris. Diets were formulated from ingredients commercially available in Egypt.

2-3-Extracts

Garlic and Artemisia vulgaris collected and dried in darkness. The air-dried and finely ground sample of each samples were extracted (Lee et al.,2004) .A500 g dry weight sample of each samples was washed, mined and added adequate amount of water to concentration of 12.5% (w/v), respectively, the ground in a blender. The extracts were passed through a 0.2 um filter. The procedures of extraction and filtration were operated at room temperature and then the sterilized filtrates were stored at 4 °C and used in antibacterial assay (Chehregani et al., 2007). In the same way control disc was also prepared by using acetone according to Asha, et al (2008) and Abdul Mannan, et al (2008). A commercial pellet feed was given once a day.

2-4-Parasitic Examination

Mucus was scraped from total surface of skin and two gill arches were removed from experimental tilapia. The degree of infestation of *Trichodina* sp. light (less than 10), moderate (10-20) and heavy (over than 20) from mucus and gills was subsequently counted under a microscope. Experimental fish were then randomly checked for parasite infections in the next two and four weeks.

2-5-Challenge experiment

After 14 days, 350 *O. niloticus* (50 in each treatment) , five fishes from each treated group and five fish from the control were examined and determined to be free from bacterial infection ,were then artificially infected by interaperitonal injection with 0.5 ml of culture suspension of pathogenic Aeromonas hydrophila containing 109 bacteria ml-1 that were previously isolateted from moribund fish. A culture suspension of Aeromonas hydrophila was prepared by culturing in agar for 24h, washed and suspended in saline (0.85%) and counted using McFarland standard tubes (No.1). The mortality (%) was recorded up to day 10 post-challenge.

2-6-Statistical analysis

Data were analyzed by analysis of variance using the SAS program (1989). Duncan's multiple-range test (1955) was used to verify significance of the mean differences among treatments.

3-Results

The examined *O. niloticus* are suffered from peticeal hemorrhage on the sides, trunk region, fins and scales. The postmortem lesion was congestion in the gills and internal organs. As well as, enlarged of gall bladder and engorged with bile Fig 1.

3-1-Parasitological results

Out of two plant extracts screened, *garlic* and *Artemisia vulgaris* had antibacterial compound against *Trichodina sp*. Showed antimicrobial activity against *Trichodina sp* as seen in Table 1.

3-2-Survival rate

Survival decreased in control group (30 %) up to 10 days after challenge infection. However, this was increased in the garlic treatment group, i.e. 60,70 and 80 % survivability in the 1,4 and 8 g garlic kg−1 respectively and 60,72 and 85 % survivability in the 1,3and 4.5 g *Artemisia vulgaris* kg−1 respectively Table 2 & 3.

Fig 1. Showing Oreomonis niloticus infested with trichodina and Aeromonua sp.

Table 1. Antiprotozo a activity of plant extracts against *Trichodina sp* in tilapia (O.niloticus)

Pond fish	**15-day during treatment**	**30-day during treatment**	**45-day during treatment**
Control	Slight	Moderate	Heavy
1g garlic	Slight	Slight	Slight
4 g garlic	Slight	Slight	……
8 g garlic	…….	…….	…….
1 g Artemisia vulgaris	Slight	Slight	Slight
3 g Artemisia vulgaris	Slight	Slight	…….
4.5g Artemisia vulgaris	……	…….	…….

Table 2. Antibacterial activity of herbal plant extracts against *Aeromonas hydrophilla*

Pond fish	**No. of fish in pond**	**15-day post treatment**	**30-day post treatment**	**45-day post treatment**	**Total No.**	**Total %**
Control (water)	50	20	10	15	35	70
1 g. Garlic	50	12	5	3	20	40
4 g garlic	50	10	3	2	15	30
8 g garlic	50	6	4	……..	10	20
1 g Artemisia vulgaris	50	12	5	2	19	38
3 g Artemisia vulgaris	50	10	5	…….	15	30
4.5 g Artemisia vulgaris	50	7	1	…….	8	16

4-Discussion

The clinical signs of heavy infested tilapia *O.niloticus* with Trichodina sp has caused gigantic financial losses, letharagic, generate excessive mucus and become off feed as recorded by Chitmanat et al,2005. The clinical infestation of challenged tilapia *O.niloticus* with Aeromonas hydrophila as peticeal hemorrhage on the peduncle region, fins, trunk and scales due to reaction of bacterial toxin and postmortem finding were congesion in gills, internal organs, these findings recorded by Cipriano,2001 who reported that A.hydrophila is highly pathogenic bacteria in the cultured and wild fish. Garlic is an important vegetable extensively cultivated in many countries. It is used as food for humans as well as some animals and as remedy for several diseases, as reported in folk medicine. Artemisia vulgarism might provide a suitable basis for new antimicrobial action Navarro et al., (1996). Otherwise the plant extracts have antimicrobial activity against fish pathogenic bacteria. Jinist (2002). Either garlic 800 mg or Artemisia vulgarism at 450 mg/kg were able to remove all

Trichodina sp. from tilapia after 2-day treatment (Table 1). All treatments were significantly different from control groups. Both garlic and Artemisia vulgaris are more economical and effective in the presence of organic matter for along time than freshly prepared Potassium permanganate for this reason, the cost of treatments would be reduced. However, Trichodina sp. became re-apparent after two weeks and act as predisposing to bacterial infection. Both garlic and Artemisia vulgarism had low acute toxicity to tilapia at the working concentration to treat Trichodinaiasis. It was found that a heavy suspension of solids adhered to the gills. However, the working concentration for *Trichodina and Aeromonus sp* treatment is much less than the concentration that causes fish deaths. Based on this outcome, either garlic or Artemisia vulgaris could be developed for safer treatment. As it is extremely desirable to reduce the use of hazardous therapeutics for *Trichodinaiasis* and *Aeromoniasis* control, there is a great potential of using garlic and Artemisia vulgaris for this treatment. All *Trichodina* sp. and signs of *Aeromoniasis* were disappeared two days after treated with either 800 ppm garlic or 450 mg Artemisia vulgaris**.** The acute toxicity response of garlic and Artemisia vulgaris to tilapia was much lower than that of Pot. permanganate. In this study, we could not use the same amount of garlic extract (200 mg/L) as shown in Madsen *et al.* (2000b) to eradicate *Trichodina* infection in eels. The possible explanation might be due to the variation of method of application and type of fish. This is one of the drawbacks of crude extract plant application. Referring to this evidence, the active ingredient for this treatment is needed to identify and find out the effective dosage before commercial application. Additionally, it is difficult to eradicate all *Trichodina* infection from the system. We found some *Trichodina* reoccurred after two week of treatment. Trichodinaiasis is primarily a problem of overstocking and poor water management; for this reason, the proper stocking density and water quality management is strongly required to relieve this problem. Madsen *et al.* (2000a) suggested that the infection pressure from Trichodinaiasis in farms with a relatively high load of organic matter may be relieved by reducing the content of organic dry matter in the processed water. Some other medicinal plants have been used as antibiotic and chemical alternatives as reported by Chitemanat t al.(2007). Results of the challenge test shown in Table 2 revealed that the mortality rate was 40,30 and 20 % with 1,4 and 8 gm/kg doses of Allium sativum and 38,25 and 15 % mortility rate with 1 ,3 and 4.5 gm/kg doses of *Artemisia vulgaris* respectively. On the other hand, the mortality rate of control was 70%. Diets with Allium sativum and *Artemisia vulgaris* showed the same effect on the mortality rate of O. niloticus challenged intraperitonealy with A. hydrophila. Allium sativum had antibacterial activity antagonized by A. hydrophila in fresh water as reported by Diab (2002), Pereira et al. (2008) and Mesalalhy et al.(2008) and Artemisia vulgaris has antiparasitic and antibacterial affect as report by Shagnliang et al,1990 and Navrro et al.(1996) who reported that methanolic plant extract from Artemisia vulgarius posses strong in vitro antimicrobial against Aeromonas salmonnicida..

5- conclusion, garlic and Artemisia vulgaris can be used as an alternatives to chemicals to treat *Trichodina and Aeromonus* sp. infections in tilapia in laboratory trials. Further studies, including the chronic effect on growth, survival rate, and reproduction need to be investigated. These results indicate that *Allium sativum* and Artemisia vulgaris has antiparasitic and antibacterial affect and makes tilapia more resistant to infection by *Trichodina sp* and *A. hydrophila*.

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Biochemical, Clinicophathlogical and Microbial Changes in Clarias Gariepinus Exposed to Pesticide Malathion and Climate Changees

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Abstract: The effect of Malathion on Biochemical changes in catfish (Clarias gariepinus) after exposure to Malathion 4.5 mg/l for 98 hours and high temperature 30◦. The obtained results showed significant increase in cooper, sodium, cortisol, urea as well as ALT and AST. It was concluded that Malathion produces metabolic stress, cell damage with malfunction of haemopoeitic system. The microbiological examination revealed presence of E-coli, Acromonas Sp, Vibrio. We can conclude that in fish reared on low CHO diet there was hyperglycemia due to increase in insulin and cortisol hormone. (Macrocytic hypochromic anemia was observed in fish in 38H and 98H treatment of Malathion. The hemogram shows increase in MCV and decrease of HB%, PCV and RBC's count. There is decrease in IgM. There was petichial haemorage in some part of skin, ascites and erosion due to complications of bacterial infections and there is vertebral column curvature syndrome. [Report and Opinion, 2009;1(6):6-11]. (ISSN 1545-4570).

Key words: Malathion Biochemical changes, Haematological changes, Microbial changes, IgM.

1. Introduction

The organophosphorous insecticide malathion (Fig. 1) is used to control pests, which attack many economic crops, (Anderson,1990). In Egypt, the Ministry of Agriculture recommended the use of malathion against pests which attack vegetable crops, ornamental plants, medicinal and aromatic plants and for protection of stored grains. Malathion is also used to control different mosquito and fly species, household insects, animal ectoparasites and human head and body lice (Roberts, 1989). The wide use of malathion is attributed to its relatively low mammalian toxicity. But like DDT and other pesticides that have been found to cause irreparable damage to human and environmental health, malathion may pose a greater risk than the product label would lead one to believe.

Shown to be mutagenic, a possible carcinogen, implicated in vision loss, causing myriad negative health effects in human and animal studies, damaging to nontarget organisms, and containing highly toxic impurities, malathion has a legacy of serious problems (Cabello *et, al.* 2001).

According to a report by the Washington, D.C. based group, *World Resources Institute* (WRI), many pesticides appear to be increasing the incidence of infections, pneumonia, ear infections, and tuberculosis. The three pesticides listed as causing this problem were DDT, malathion, and the pesticide aldicarb (Breener, 1992).

The environmental protection agency (EPA) has been stating for years that they would require more detailed tests for chemical effects upon the immune and nervous system. However, to date, these requirements have not been implemented. Perhaps the biggest unknown risk from malathion is its potential to increase risk of contracting bacteria or viral infections such as encephalitis, this paradoxical situation arises since exposure to malathion can weaken a person's immune system (Giri *et, al*. 2001).

Other effects of malathion for which there is no research, but seriously needed include its ability to cause: Learning disabilities, short term memory damage, increase risk of allergies (Cabello *et, al.* 2001).

Research has accumulated which indicates that nutritional factors can significantly modify the host response to environmental toxicants. Correction of malnutrition can clearly mitigate the effects of many toxicants; however, evidence is mounting that supraphysiologic doses of nutrients (nutritional supplements) can further lessen toxicity. The possibility that nutrition could be implemented as a secondary

prevention strategy on a public health scale raises important ethical and policy issues. Nutritional strategies can lessen, but not abolish, toxic effects; moreover, they require dissemination and compliance, which are unlikely to be fully effective (Hu *et, al.*1995).

Malathion accumulate in fish mainly in the visceral fat, where as the gills and muscles retain a lower amount subsequently, with an increase in fat consumption. For example, at the time of migration and hibernation, pesticides may enter the more sensitive organs and induce poisoning (Bruno and Stamps, 1987), (Barton and Iwama, 1991), (Bennett and Wolke, 1987) (Pickering and Duston, 1983). It has been presumed for decades that environmental pollutants especially pesticides can affect one or more of the immunological functions in the fish. It is almost common knowledge cat fish frequently then become more susceptible to various diseases given the extreme variety of pesticides used (Vergut and Studnicka, 1994), (Areechon and Plumb, 2000). Andreson, 1990 suggested a decreased disease resistance in fish exposed to various pesticides. There is so little is known about how pesticides affect the immune systems of fishes (Cabello *et, al.* 2001).

CH_3 O P - S – CH – CO_2– C_2H_5
CH_3 O CH_2 – CO_2 – C_2H_5

Fig. 1: "Malathion"

The chemical name is : S-1 2 bis (ethoxycaronyl) ethyl 0,0-dimethyl-phoshordithioate (IUPAC).
-Trade names : Malathion, Cythion, Fyfanon and Calmathion (Royal Society of Chemistry, 1993).

The present study discusses the effect of low CHO diet on cat fish, which also exposed to Malathion (4.5 mg/l) as pesticide for 98H. Some microbiological and clincopathological parameters were interpreted.

2-Material and methods

Experimental condition: Catfish (50 – 60 gram/each) were obtained from River Nile Rashid branch, El-Kanater El-Khyria. Fish were acclimatized to laboratory conditions one week before infection in 115 L. glass aquaria with a flow system and dechlorinated tap water. Two groups of fishes were used.

The 1st group (15 fishes) was maintained kept on low carbohydrate diet but free from any toxicants, and kept on a balanced diet that meets its requirements from nutrients as described by (Robberts, 1989).

The 2nd group (15 fishes) were kept under the low CHO diet but were exposed to Malathion (4.5 mg/l) during 98 H hours (Areechon and plumb 1990). The diet ingredient is shown in (Table 1).

Copper, dissolved oxygen, temperature, pH, ammonia and nitrites were analyzed daily, while water alkalinity, hardness carbon dioxide, sodium, potassium and chlorides were analyzed before and after each water renewal using commercial kits of Bohringer, France, as shown in (Table 2). Malathion was obtained from National Institute of Pesticide, Dokki, Cairo. The 98h LC50 of Malathion for channel cat fish determined in separate study was 9.65 mg (Areechon and Plumb, 1990).

Table (1): Ingredients and proximate chemical composition of diets used in experiments.

Ingredients	Diets I (control)	Diets 2
Fish meal	30	30
Meat meal	8	10
Bone meal	1	3
Skimmed milk	3	4
Soybean	5	7
Wheat bran	20	20
Wheat flour	20	5
Yeast	10	15
God liver oil	1	4
Minenral & Vitamin* premix	**2**	2

Proximate chemical composition:

Crude protein (CP) %	35.87	38.89
Metabolizable	2297.21	2415.4
energy/kg	2.78	2.86
Ether extract (EE) %	3.91	4.27
Grude fiber (CF) %	8.735	10.25
Ash %	3.094	3.99
Calcium (Ca) %	2.069	2.53
Phosphorous (Ph) %	2.105	2.29
Lysine %	0.562	0.613
Methionine %		

*** Mineral and vitamin premix per/kg of pelleted food :**

Vit.A, 8000 U; Vit. D, 9001, Vit. E 21 U, vit. K, 4 mg; Vit. B2 3.6 mg; niacin 20 mg choline chloride, 160 mg; pantothenic acid, 7 mg; pyridoxine, 0.2 mg; Vit. B 12, 5 ug; Mn, 70 mg, Zn 60 mg, Fe 20 mg, Cu 2 mg, I 1 mg, Co 0.2 mg.

Blood sampling :

Blood samples were taken after 24h, 38h, 98h. The fish were anaesthetized by 1/1000 aqueous solution of Ms 222 and bled from the caudal vein. Blood samples were taken with heparinized microhaematocrit

tube. The tubes were centrifuged at 3000 r.p.m. for 10 min. Serum was separated and stored at 20°C until used.

Table (2) : Water quality characteristics in tanks. Initial conditions values are mean ± SE.

pH	5.40 ± 0.1
Temperature °C	18°C ± 0.904
Nitrates mg/l	0.020 ± 0.04
Un ionized ammonia (mg/l)	0.0014 ± 0.004
Carbonic dioxide (mg/l)	4.1 ± 0.5
Alkalinity (mg/l)	31.8 ± 2.8
Permanganate oxidabole matter (mg/l)	3.54 ± 0.53
Hardness (mg/l)	34.6 ± 0.1
Chlorides (mg)	8.4 ± 0.6
Potassium (mg/l)	0.12 ± 0.007
Sodium (mg/l)	5.68 ± 0.01

Tested kits supplied form biomerieux (France) were used for determination of the activity of serum glutamic pyruvic transaminase (ALT) and glutamic oxaloacetic transaminase (AST) as described by (Reitman and Frankel, 1957).Serum glucose was assessed according to (Trinder, 1969). Haematocrit value was carried out by using micro-haematocrite capillary tubes centrifuged at 1200 r.p.m. for 5 min. mean corpuscular volume (MCV). Reticulocytis count according to (Drabkin, 1946). Serum cortisol level was determined using radioimmunoassay technique according to the method of (Pickering and Pottinger, 1983). Serum iron were determined using atomic absorption according to (Barham *et al*., 1972). Values of sodium and potassium in serum were determined by flame photometer according to method described by (Silversmit, 1965). Serum creatinine was measured according to (Bartels *et al*., 1972). Enzymatic determination of urea was done according to (Patton and Crouch, 1988). Insulin was estimated by radioimmunoassay method using oat. A Cout insulin Kits obtained from Diagnostic Corporation (DPC) west 96th street, Los Ageles U.S.A. (Pickering and Duston, 1983).

Bacterial Isolation:

Aseptic swabs from the skingills, base of fins and blood of tested fish were cultivated on blood agar, MacConky agar, Nutrient agar, TSA, Nutrient broth, and peptone water (Oxoid and Difco).

Inoculated media were incubated at 37°C for 48 hours. Bacterial isolates were identified by examination of the colony morphology and biochemical characteristic described by (Nagae *et al*., 1993). Bacteria were detected by accounting colonies using surface spread plate technique according to quantitative method described by (Bruno & Stamps 1987).

Measurement of serum immunoglobulin M (IgM) :

IgM determination :

The serum IgM was measured according to (Fuda *et al.* 1991).

Preparation of antisera :

Antisera of cat fish was prepared by immunizing rabbits as described by (Hara, 1976).

(Cat fish) IgM antibody :

The procedure for labeling antibody of fragment with enzyme was performed according to the method of (Nagae *et al.*, 1993).

Elisa assay procedure :

Assays were carried out in 96 well polystyrene ELISA microtiter plates (Titertex, Horsham, PA).

Antibody coating :

The micortiter plates were coated with rabbit Anticat fish IgM which was fractionated by DE-52 at a concentration of 40 ug/ml in 0.01 M PBS. A volume of 150 µl was dispensed into each well and incubated for 4 hr at 4°.

Blocking :

After one washing with 200 µl of 0.01 M PBS + 0.1 % Tween 20 per well and two washings with 200 µl of PBS + 1 % thimerosol was added to each well and included for 2hr at room temperature.

Incubation of samples and standards:

After washing as described above 100 µl of sample and standard were placed into the appropriate wells in the microtiterplates and incubated at room temperature.

Incubation with peroxidase labeled antibody:

After washings as described above, each well received 150 µl of peroxidase labeled antibody 1:1600 in PBS-BSA, followed by incubation 12 hr at room temperature.

Enzymatic color reaction:

The plates were washed as described above and 150 µl 0-phenylenediamine (3 mg/ml 0.1 M citric acid-phosphate buffer (pH 5.0) containing 0.02 % H2O2 were added to each well for enzymatic color reaction. The reaction was stopped after 30 min at room temperature by adding 100 µl of 4NHCI. The absorbance at 492 nm was 2250 (Richmond, CA).

Double antibody sandwish Elisa according to the method of (Matsubara *et al.* 1985) for determination of IgM described. After one washing with 200 µl of 0.01

M PBS + 0.1 % Tween 20 per well.

3-Statistical analysis:

The obtained data were statistically subjected to the students't-test (Gad and Weil, 1983).

4-Results :

Experimental exposure to Malathion (4.5 mg/l) revealed that there was a significant increase in the level of serum creatinine ALT, AST, urea, potassium and insulin, were increased in the 24 hours and 38 h and 98 h non-significantly. There was a significantly increase of cortisol, glucose, copper during all times of experiments. Concerning iron there was a significant decrease of iron level (Table 3).

Hematological results in the present work revealed anemia indicated by a significant reduction in RBCs count, HB concentration PCV %, MCV and increase in reticulocyte count especially in 38 h, (Table 4).

Concerning microbiological examination, the results showed that the isolated microorganisms from internal organs (liver and kidneys), gills, and fins were Aeromonas Vibrio Sp and E. coli. (Table 5). With regard to IgM, a significant decrease was observed by exposing cat fish to the pesticide. (Table 3).

There was petichial haemorhage in some part of skin and erosion due to complications of bacterial infection and vertebral column curvature syndrome.

5-Discussion:

It was evident that decrease in the level of CHO with Malathion 4.5 mg/l in fish diet caused a significant increase in glucose level during the experimental period, also insulin level was slightly increased. It is well known that any stress factor such as handling, incubation, anaestesia etc. has been shown to cause hyperglycemia followed by hyperinsulinemia (Yallow and Bawman, 1983).

Low CHO diet with Malathion causes a significant increase of cortisol level which may be due to the activation of hypothalamus, pituitary internal axis. Induced a significant increase in cortisol level, these results coincide with those observed by (Barton and Iwama, 1991), who observed that serum cortisol increased linearly in salminid fish fed on 5% CHO diet.

One consistent effect of cortisol was the reduction in the hemoglobin, PCV % and iron levels, as a result of decrease in appetite in the rainbow trout, or more likely to be the direct result of a catabolic effect, or cortisol of the fish tissues.

This present study revealed that, sodium and potassium concentrations were significantly increased. This retention may be attributable to kidney impairment where the kidney is the normal pass way for Na and K this may explain the main cause for elevation of the serum creatinine and urea in the treated groups.

Marked elevation was noticed in the activity of Asparate Amino Transferase (AST) and Alanine Amino Transferase (ALT). The liver is the primary organ of detoxification as well as a major site for detoxification reaction. Therefore, significant increase in the liver enzymes suggests explanation Malathion affected the liver cells or may be attributed to secondary bacterial infection.

The present results agree with (Bruno & Stamps 1987) they observed that aquatic pollution with heavy metals cause immunosuppression and contribute to outbreaks of infections, and bacterial diseases in fish. We can say that Malathion can affect fish after 24h and there are some complications with this pesticide.

IgM level was determined to find out information about fish immune system, which was previously investigated in different species by many authors as (Matsubara *et al*., 1985) and (Fuda *et al.*, 1991).

There is a significant decrease in IgM level in fish with Malathion, if compared with control groups. Anderson *et al.,* 1982 found a relation between corisol and IgM as when cortisol increased IgM decrease.

IgM is one of the most important factors in the immune factor to neutralize bacteria and render them more susceptible to phagocytosis (Mona S. Zaki *et al.,* 2003). It is well known that in mammals immunoglobulin production is closely related to endocrine status for example thyroid hormone enhances the production of immunoglobulin (Chen, 1980) cortisol intensity suppress immunoglobulins production (Pickering & Pottinger, 1983).

In conclusions Malathion will reduce humoral immune response as detected by decrease of IgM level and cortisol elevation.

6-Akhnowalgment

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Table (3): Effect of low CHO diet on some biochemical and hormonal parameters in cat fish exposed to Malathion (4.5 mg/l).

Parameters	Control	24 hours	38 hours	98 hours
AST (U/I)	77.0 ±0.53	80.7 ±0.60	83 ± 0.51	100±0.85**
ALT (U/I)	15.3 ± 0.33	17.4 ±0.24	18.9 ±0.83	23.0±0.83*
Urea (mg/dl)	4.3 ± 0.51	4.9 ± 0.76	4.9 ±0.83	5.1±0.83*
Creatinine (mg/dl)	0.67 ± 0.62	0.69 ± 0.72	0.90±0.51*	1.3±0.53*
Na (Meq/L)	117 ± 1.3	127 ± 2.3*	128 ± 4.5*	142±5.4*
K (Meq/L)	2.58 ± 0.12	3.65 ± 0.13	1.3 ± 0.8	5.00±0.80*
Cortisol (ng/dl)	0.85 ± 0.23	0.88 ± 0.54	0.91 ± 0.39	1.5±0.45**
Glucose (mg/dl)	52 ± 0.59	54.1 ± 0.50	68 ± 0.59	85±0.23**
Insulin (ng)	7.6 ± 0.3	8.5 ± 0.4	11.7 ± 2.4	12.8±0.63**
Copper (mg %)	181 ± 4.0	187 ± 2.3	168 ±1.3	148±0.45**
Iron (mg %)	190 ± 1.26	176 ± 5.2	168 ± 3.3	151±4.3*
IgM Ng/MI	0.85 ± 1.32	0.78 ± 1.20	0.63 ± 0.60	0.65±0.44*

*P < 0.01 **P < 0.05

Table (4): Effect of low CHO diet on hematological parameters in fish exposed to malathion (4.5 mg/l) for 98 H.

Time Groups	**Parameters**				
	RBCs (106/mm3)	HB gm/dl	P.V.C.(%)	MCV Fl.	Reticulocyte %
Control	3.2±0.34	7.7 ±033	8.7 ± 0.33	31± 0.51	1.21±0.2
24 hours	3.3±0.34	8.6 ± 0.10	8.6 ± 0.11	37 ±0.41	1.92±0.3
38 hours	3.9±0.63	8.7±0.17*	7.87±0.11	38±0.73	2.3±0.4
98 hours	3.7±0.62	8.3±0.28*	6.3±0.37*	39±0.83*	2.4±0.4*

*P < 0.01

Table (5): Bacterial isolates recovered from fish exposed to Malathion (4.5 mg/l).

Bacterial strain	**External surface**	**Kidneys**	**Liver**	**Gills**
E. Coli	2 X 10	3 X 10	2 X 10	1 X 10
Aeromons Sp.	3 X 10	6 X 10	3 X 10	2 X 10
Vibrio/ Sp.	2 X 10	1 X 10	2 X 10	2 X 10

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Acetylation of Some Azo Dyes and Its Effects on the Thermodynamic Parameter, Colour and Fading Values on Nylon 6, 6 and Wool Fabric.

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ABSTRACT

Azo dyes containing free amino group were derived from H-acid and the free amino group was acetylated to obtain another group of dyes. The two groups of dyes were applied to wool and nylon 6.6 fabrics and their colour fastness properties assessed. It was found that acetylated dyes gave hypsochromic effects on colour, and dyeing fastness such as washing and perspiration improved on acetylated dyes when compared with unacetylated dyes on both fabrics. For the light fastness, however, there was no noticeable improvement on nylon 6.6 fabric but general improvement was made on acetylated dyes when applied to wool fabric. For the thermodynamic parameters such as the equilibrium constant (partition coefficient K) and the standard affinities $\Delta\mu^0$ the acetylated dyes have lower values when compared with the unacetylated dyes on fabrics. [Report and Opinion, 2009;1(6):12-17]. (ISSN 1545-4570).

Keywords: Azo dyes, Hypsochromic, Unacetylated dyes, Nylon 6, 6, Standard affinities.

1. Introduction

Light and weather fastness is an important factor in the determination of the useful life span of textiles. Light may produce two different changes in dyed fibrous materials; on the one hand the colour can fade, on the other hand photochemical degradation of the fibrous material can take place. It is well known that certain yellow and orange vat dyes sensitizes the photodegradation fo cellulosic fibres such as cotton while acid metal-complex dyes serve to protect nylon fibres [1,] (Kricserskz kij 1975) Baumann[2] (1970) concluded for experimental results obtained from amino acids that certain reactive dyes linked to the side chain slow down the photodegradation of wool, while they promote reaction when attached to the principal peptide chain.

It has also been reported that acetylation of free amino groups of some dyes lead to an improvement in the light fastness to such an extent as much as 3-4 units on wool fabrics[3] (Okubu 1953) and it has been proved that both $-NH_2$ and –OH groups are the source of fugitiveness in the dyes containing them[3] (Okubu 1953). It was therefore considered of interest to study the effects of acetylating only the free amino group contained in acid dyes derived from H-acid to study the change in the thermodynamic value and effects on fading behaviours of the resulting dyes on wool and nylon 6.6, the –OH group is left intact. The general formula of the azo dyes used in this study is given in Fig. 1.

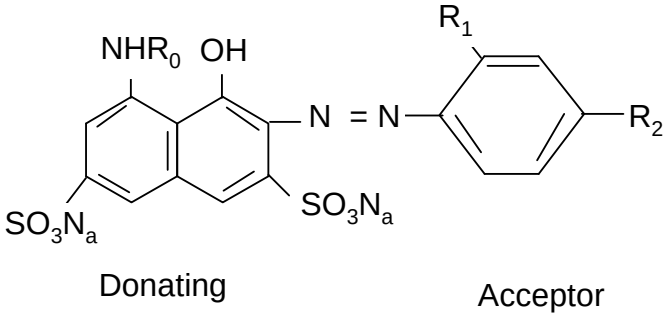

Fig. 1: General formula of the azo dye used in the study

Dye No.	Substituents		
	R_0	R_1	R_2
1	-H	-H	-COOH
2	$-COCH_3$	-H	-COOH
3	-H	-CN	$-NO_2$
4	$-COCH_3$	-CN	$-NO_2$
5	-H	-C1	$-NO_2$
6	$-COCH_3$	-C1	$-NO_2$
7	-H	$-NO_2$	-C1
8	$-COCH_3$	$-NO_2$	-C1

2. Materials and Methods

2.1 Experimental Acetylation of H-acid

1g (0.0033 moles) of H-acid was dissolved at 50^0C in 7.00cm^3 of water containing (0.0019 moles) 0.2g of soda ash in a three necked round bottom flask fitted with a mechanical stirrer, reflux condenser and thermometer. With vigorous stirring (0.006 moles) 0.58g of acetic anhydride was added into the flask over a period of 15 min. strirring was continued for at least one hour to ensure complete acetylation of the H-acid. After complete acetylation, (0.02 moles) 1.66g of soda ash was added to the reaction mixture to hydrolyse off any acetyl group that may have substituted the hydrogen atom in the hydroxyl group of the H-acid.

2.2 Dyes

The alkaline solution of acetyl H-acid obtained above was used directly in the coupling reaction with the proper N, N-dialkylaniline according to usual procedures[4] (Fieize-mild 1949). The impure dyes were recrystallised from alcohol and water mixture and their purity was controlled by TLC.

2.3 Physico-chemical properties

Visible spectra of the dyes were obtained with a specord u.v.-vis spectrophotometer using water solutions at concentrations of 10^{-4}-10^{-5} mol/1. The results obtained are summarized in Table 1.

2.4 Dyeing procedure

Wool and nylon fabric samples were dyed in a Waterman dyeing machine. The liquor ratio was 50:1 at pH 3. The dyeing was carried out with the samples entered at 45^0C to 100^0C over 15 minutes and maintained at this temperature for 45 minutes. When the dyeing was complete the temperature was lowered and the samples were rinsed and washed under standard conditions [7].

2.5 Measurement of % Exhaustion

The exhaustion of the dye was determined by measurements of optical density using a colorimeter at the λ_{max} of each dye. The optical density of the dyebath was measured before the commencement of dyeing by taking a sample from a control dyebath. At the completion of dyeing, the dyed sample was removed and excess dye liquor squeezed back into the dyebath. The dye liquor was cooled, made up to the initial volume with distilled water, a sample was taken and its optical density measured. Percentage exhaustion was calculated using the relationship below. Duplicate measurement was made and the average exhaustion value taken.

$$\%Exhaustion = \frac{(InitialO.D - FinalO.D)}{Initialo.D} x100$$

Table 1: Visible Absorption Spectra for the Dyes

Dye No.	λmax (water) (nm)	ε_{max} x 10^{-4} $1/mol^{-1}cm^{-1}$	Δλa* (nm)
1	539	2.39	
2	510	1.85	29
3	569	1.55	
4	499	1.26	70
5	577	1.72	
6	515	1.85	42
7	541	1.37	
8	495	1.17	46

*Δλ = (λmax unacetylated dye – λmax acetylated dye)

2.6. Wash fastness test

The washing fastness test was carried out using a Linitest was wheel machine following ISO procedure[5] (SOC 1978). In this case, soap solution was prepared containing 5g/1 soap and 2g/1 Na carbonate in distilled was cut. Each of the dyed fabrics was two adjacent white fabrics of same dimensions and stitched together. For the wool fabric, one of the adjacent fabrics was made of wool, while second was cotton. Also, in case of nylon 6.6, one of the adjacent fabrics was cotton, which the other was nylon 6.6. The composite was then placed in the container of the Linitest machine and necessary amount of sopa solution previous heated to 60 ± 2^0C added to a liquor ratio of 50:1. Washing was carried out at this temperature for 30 min. The compostion were then removed, rinsed opened, dried and finally assessed with the aid of gey scales. The results obtained as summarized in Table 2.

2.7. Acid and Alkaline Perspiration Tests

Composite specimens of the dyed samples were prepared as for the wash fastness test. These were then treated in freshly prepared alkaline and acid perspiration test solutions. The artificial perspiration solution used in this study were perepared in the following ways:

(a) Alkaline perspiration solution 5g/1 sodium chloride 0.5g/1 Histidine monohydrochliride monohydrateThe solution was brought to pH 8.0 with 0.1N solution of sodium hydroxide.

(b) Acid perspiration solutions 5g/l sodium chloride 2.2g/l disodium hydrogen orthophate 0.5g histidine monohydrochloride phydrate. The solution was brought to pH 5.5 with 0.1N sodium hydroxide. The composite sample was improved in he artificial perspiration solutions separately with liquor ratio of 20:1 for 30 min.

Table 2: Fastness properties of the Dyes on Nylon 6.6 fabric

Dye	Wash change in shade	Fastness degree staining in	Perspiration Alkali (a)	(b)	Fastness Acid (a)	(b)	Light fastness	% Exhaustion
1	3	5	4	4	4-5	4-5	2	71.2
2	4	5	3-4	3	4-5	4	2-3	51.5
3	2	5	2-3	5	4-5	3	3-4	87.9
								39.4
4	4-5	5	4	4-5	3	4	2	
5	2-3	5	2	4-5	2	4-5	4	71.2
								60.6
6	3	5	3-4	5	5	5	2	
7	2-3	5	2-3	3	2-3	4-5	2	76.7
8	4-5	5	2-3	5	2-3	5	1	66.7

Change in shade (a)
Staining of adjacent white material (b)

Table 3: Percentage Exhaustion and fastness properties of the dyes on wool

Dye No	% Exhaustion	Light fastness	Wash (a)	Fastness (b)
1	92.8	6	4	5
2	97.2	7	4-5	5
3	98.4	3	3	4-5
4	96.0	4	3-4	5
5	99.2	4	4	4-5
6	98.9	5-6	4-5	4-5
7	99.4	6	3-4	5
8	98.6	7	3-4	5

a = Change in shade
b = Staining of adjacent white material

Table 4: Thermodynamic Parameters (K and $\Delta\mu^0$) on Nylon 6, 6

Dye	% E	Partition coefficient K (ℓg^{-1})	Standard affinity $\Delta\mu^0$ (kJ mol^{-1})
1	71.2	123.6	14.94
2	51.5	53.1	12.32
3	87.9	363.2	18.28
4	39.4	32.5	10.79
5	71.2	123.6	14.94
6	60.6	76.8	13.47
7	76.7	164.5	15.83
8	66.7	100.2	14.29

The composite was removed and arranged between glass plates one on top of another and a weight of 4.5kg placed on top. The entire assembly was then placed in an oven at 37 ± 2^0C for 4 hours. The assembly was then removed after four hours from the oven, and the composites were then dismantled and air dried at temperature not exceeding 60^0C. The degree of perspiration fastness was judged using a grey scale for assessing colour change and staining of adjacent materials.

2.8. Light Fastness Test

An artificial light source (exnon arc) was used to determine the light fastness following procedure described elsewhere[6].

3.0. Results and Discussion

3.1. Light Absorption Properties

The visible absorption property of the dyes was measured in water and the molar absorption coefficient calculated for the same solvent, the results obtained are summarized in Table 1. The dye can be classified as donor-acceptor chromogen in which the phenyl ring is serving as electron acceptor half and the naphthalene ring is acting as the electron donating half of the chromogen. For example dye (1) which contains the carboxylic group as electron withdrawing subsistent in the acceptor half absorbs at 539nm in water with molar extinction coefficient of 2.39 x 10^{-4} 1/mol-cm^{-1}. When the electron donating amino group in dye (1) is substituted with acetyl group as in dye (2), the resulting dye (2) absorbs at 510nm which is at a shorter wavelength of 29nm relative to dye (1). In this case the acetyl group has reduced the electron donating property of the amino group in dye (1) and thus caused an hypsochromic effect of 29nm.

In dye (3) the carboxylic group in the parent dye (1) has been substituted with nitro group and additional electron withdrawing cyano group was introduced in the acceptor group ortho to the azo group, and in this case the dye (3), absorbs at relatively longer wavelength at 569 nm when compared with dye (1). This is expected as a result of two powerful electron withdrawing groups substituted in the acceptor half of the chromogen. This has actually resulted in bathochromic shift of 30nm relative to dye (1). The acetylation of the amino group in dye (3) to give (4) also results in hypsochromic shift of 70nm relative to dye (3). This is consistent with the result obtained when dye (1) is acetylated to give

(2). The acetyl group is in effect an electron withdrawing group substituted in the electron donor half of the chromogen.

The results obtained with dyes (5) and (6) also follow the same trend, as can be seen from Table 1. dye (5) absorbs at 557 nm whereas, dye (6) in which the amino group has been acetylated absorbs at 515 nm, showing hypsochromic shift of 42 nm in dye (7) and (8), the relative positions of chlorine and nitro groups are interchanged from the positions in which they were in dye (5) and (6). When the corresponding pairs of the two dyes are compared, it can be seen that nitro group in the para position to the azo group gave dyes which are more bathochromic than the corresponding dyes with chloro group in the same position. For example dye (5) absorbs at 557nm whereas substitution of nitro group with chloro group to give dye (7) absorbs at 541nm, a hypsochromic shift of 16nm. The same effect is observed when dye (6) and (8) are compared.

3.2. Thermodynamic Parameters

For the thermodynamic parameter the trend followed the same pattern observed in the various λmax. For instance dye 1 which has the carboxylic group as electron withdrawing substituent in the acceptor half absorbs at 539nm and has standard affinity of 14.94kJmol^{-1} whereas dye 2 having acetyl group in position of amino group absorbs at 519nm, and standard affinity of 12.32kJmol^{-1}. It implies that dye 2 absorbs at a shorter wavelength of 29nm relative to dye 1, and 2.62kJmol^{-1} of standard affinity (energy) less than that of dye 1. This is the trend between dyes that were acetylated and those that were unacetylated (Table 4). All the 'odd' numbered dyes were unacetylated, while all the 'even' numbered dyes were acetylated.

For dyes 3 and 4, the results obtained in their % exhaustion can be explained on the platform of the fact that both CN^- and $–NO_2$ are electron withdrawing group and this enhanced the release of a lone pair of electrons on amino group (R^o.) i.e. protonation easily occurred there and this positively affect dye uptake by polyamide fabrics such as nylon and wool, hence very high equilibrium exhaustion (87.9 %). On the other hand, dye 4 that is acetylated is not easily protonated, despite the presence of –CN and $–NO_2$ groups that are electron withdrawing groups, hence very low equilibrium exhaustion (39.4 %). The same thing applies to dyes 5 and 6 even though to a lesser magnitude compared with the pair dyes 3 and 4.

The results summarized in table 1, generally show that acetylation of amino group in the azo dyes under study lead to hypsochromic effects when the corresponding pairs of dyes are compared. This is due to the fact that the strength of the electron donating half of the chromogen has been reduced when the acetyl group is placed at that position. When the relative strength of the electron withdrawing groups incorporated in the acceptor half of the chromogen is compared, the nitro group para to the azo group influenced a powerful electron withdrawing strength, though with the aid of cyano group ortho to the azo group which gives the most bathochromic dye in the series.

3.3. Dyeing fastness

The results of the I.S.O. No. 3 wash fastness test summarized in Table 2 and 3 shows that all the dyes gave dyeing of excellent wet fastness on nylon 6.6 materials, however, the wet fastness ratings obtained with the dyeing of the dyes on wool are not as good as on nylon 6.6. This is due to the fact that wool fabric absorbed more dyes as indicated by the percentage exhaustion in Table 3 than nylon 6.6 as in Table 2 and in this way more dyes will be desorbed out of wool during the washing treatment. The results of the perspiration test also show that both the alkaline and acid perspiration fastness is good on nylon 6.6 materials and it can not be seen whether the acetylation has any effect on this fastness property.

The results of light fastness test summarized in Table 3 for wool fabrics clearly show the effects of acetylation on light fastness property. When all the dyes are compared on wool, it is seen that acetylated dyes have good resistance to light than the unacetylated dyes. All the dye that contain free amino group gave reduced light fastness property. Free amino group in dyes increases the surface activity of the molecule and consequently reduces its photo-stability. The nature of the dye molecule allows the dye molecule to react with surrounding materials such as free oxygen in the air when some form of light is absorbed. In this respect, the dye molecule decomposes and loses its colour. The activity of the free amino groups in dyes 1, 3, 7 and 9 have been reduced by acetylation and these account for the improved light fastness for other dyes with acetyl group in the dyes under study.

When the light fastness properties of the dyes under study are compared on both nylon 6, 6 and wool fabrics, the dyes have better light fastness on wool than on nylon 6, 6. It is possible however, that particular amino acid residues in wool retard photo-oxidation of the dye by reacting preferentially with oxidants generated on exposure of the dye fiber. Another reason for increased light fastness of the dye on wool can be seen from the percentage exhaustion of dye on both fabrics. The dyes are generally

more exhausted on wool than on nylon 6, 6, and therefore the amount of dyes contain by wool fabric in each case of the dyes are more and for this reason, light destruction will take a very long period before it can be noticed on wool being that they are present larger amount than on nylon 6, 6.

From the results of light fastness properties there is no improvement on nylon 6.6 when the dyes are acetylated and this show that different mechanism of fading on nylon 6, 6. It is well known however, that resistance of a dye or pigment to chemical in photochemical attack is directly related to the chemical structure and physical characteristics of the fiber itself.

4.0. Conclusion

The results of the study clearly show that acetylation of the amino group leads to hypsochromic effect in terms of colour and constitution study. By extension the partition coefficient and standard affinities (energies) of the dyeing which are the driving forces behind dyeing process. Also acetylated dyes enhanced light fastness properties on wool but not on nylon 6,6. It is strongly suspected that there is an empirical relationship between the thermodynamic parameter of dyeing such as partition coefficient K, standard affinity $\Delta\mu^{\theta}$, heat of dyeing ΔH^{θ} and indeed, the entropy of dyeing ΔS^{θ} and then where a particular dye has its λ_{max}. This is still under investigation.

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Assessment of Top Soil Quality in The Vicinity of Subsided Area in the South Eastern Part of Jharia Coalfield, Jharkhand, India

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Abstract: Coal mining is the major mining activity performed in the India. There are two types of methods applied for mining such as opencast mining and underground mining. Both produce huge amount of wastes, especially while performing the opencast mining activities. Opencast project involved displacement of large amount of overburden to excavate the valuable mineral. In coal mining areas the top soils are affected by various activities such as blasting, drilling and quantity of explosives used. Coal mining operations have resulted in environmental degradation, loss of soil fertility, pollution of water source and ecological changes. This paper presents the results of the studied carried out on top soil (0-15cm) quality parameters of South – Eastern part of Jharia coalfield. It has been observed that the soil quality in subsided areas have relatively low pH, low moisture content and high conductivity. The present study of top soil quality in subsided areas and its results are very vital for planning the rehabilitation programmed of Jharia coalfield, Jharkhand state.[Report and Opinion 2009;1(6):18-23]. (ISSN: 1553-9873).

Key Words: Opencast project, Top soil,, Overburden materials, Subsided areas.

1. Introduction Topsoil is the upper surface of the earth's crust, and usually is no deeper than approximately eight inches (20 centimeters). The earth's topsoil mixes rich humus with minerals and composted material, resulting in a nutritious substrate for plants and trees. It may one of the earth's most vital resources, because it represents a delicate nutritional balance that provides food for many of the animals on earth, either directly in the form of plant material or indirectly in the form of products from animals that eat plants. Topsoil is formed over a long period of time. Soil formation takes place when many things interact, such as air, water, plant life, animal life, rocks, and chemicals. The topsoil will be dark brown or black in color (though not in very arid or dry areas), and is made up of rock material that has been chemically and physically broken down and changed, and mixed with organic materials such as dead plants, particularly roots of them. Furthermore, top soil will generally be full of plant and animal life.

In coal mining areas, the top soils are not selectively handled as a result the top soil material gets mixed with overburden materials and thus the soil, an important resource for land management is lost and it degrades the quality of agricultural soil.

Mining activity in Jharia coalfield (JCF) started in 1895 and large scale mining started in 1920. Mining activity includes mainly opencast mines grew rapidly due to better quality of coal and multi seam occurrence in this field. Opencast mines were owned by local or private owners operating in very small patches or small units (Sharma, B.K, 2007). During this period (Nationalization of coal industry, 1971 - 73), coal was exploited by the local or private owners in an unscientific way. This led to many problems such as loss of livelihood, loss of habitation, loss of human health, subsidence and fire problems which create havoc in many localities. Most of the problems of subsidence and fire are scattered throughout the Jharia coalfield especially south eastern part of JCF. These problems are mainly concentrated along the eastern side of the

JCF viz. Lodna area, Kustore area and Bastacolla area. The fires have affected surface structures, houses, buildings, and top soil in its vicinity. Top soil varied in their composition and properties depending upon the fire and subsidence involved. The subsidence movements cause the changes in the surface topography and surface drainage system (Saxena et al., 2000). These subsidence movements can damage all the categories of the surface properties around the subsided areas. In Figure 1 fire affected area has been shown.

Figure 1. Fire affected area in south eastern part of Jharia coalfield.

2. Materials and Methods

2.1Study area

The Jharia Coalfield (JCF) is one of the Lower Gondwana Coalfields of India, covering an area of about 72 km^2. It is one of the most important coalfields in India, located in Dhanbad district, between latitude 23° 39' to 23° 48' N and longitude 86° 11' to 86° 27' E. The coalfield is named after the chief mining centre Jharia which is situated in the eastern part of the field.

This is the most exploited coalfield because of available metallurgical grade coal reserves. This sickle shaped coalfield is about 40 km in length and approximately 12 km in width stretches from west to east and finally turns southward covering an area of about450 sq.km. It is sickle shaped coalfield occurring in the form of basin and truncated with a major fault, known as boundary fault, on the southern flank. The average thickness of coal seams in the coalfield in the depth range upto 600 meters could be about 100 meters. The general map of Jharia coalfield is shown in figure 2. Sampling sites has not been mentioned in this map.

JCF is located about 260 km North West of Kolkata in East India. JCF contains 40 identified coal horizons and the leasehold area of Bharat Coking Coal Limited (BCCL) is 270 sq.kms (Sharma, B.K. 2007).This coalfield has one of the highest coal densities in the world.

For the purpose of conducting various tests on the top soil in the laboratory, soil samples have been collected from selected subsided zone viz. Lodna area, in Jharia coalfield. Lodna area is fire affected area. Removal of top soil due to mining affects soil properties mainly through the loss of soil organic matter, plant nutrients and exposure of sub soil material with low fertility and high acidity.

2.2 Soil samples

The air dried top soil samples were ground and pass through 2mm sieve. The care must be taken to collect representative samples and record the samples so that lab results can be used. Soil should be collected with a clean soil sampling tube. Debris should be cleared from the surface and a hole dug to six inches. Once the hole is dug, scoop a part of soil off one side of the sampling tube and place the part of soil into a clean polythene bag. Top soils samples can be drawn with any of these equipments namely, soil auger (tube), screw type auger, spade, gardening hand tool (Khurpi). The collected top soil samples after coning and quartering then sieving (2mm) were used for analysis of different soil quality parameters.

2.3 Sampling sites and analysis

Lodna area of BCCL is situated in the south eastern part of JCF. It is the one of the most important colliery and subsided zone in Jharia coalfield, Dhanbad, Jharkhand. Some of the colliery of Lodna Area is facing serious subsidence problems due to presence of small patches of underground fires scattered throughout the area. In Table 1 sampling sites, longitude, and latitude are mentioned. Opencast cast mining has also been done in this area

such as North Tisra. Representative composite top soil samples were taken from the Lodna area of JCF up to 15 cm depth as per standard procedure of Indian agricultural research institute, New Delhi. The location of sampling sites was randomized to avoid biasing in results. In figure 3 degradation of top soil is shown.

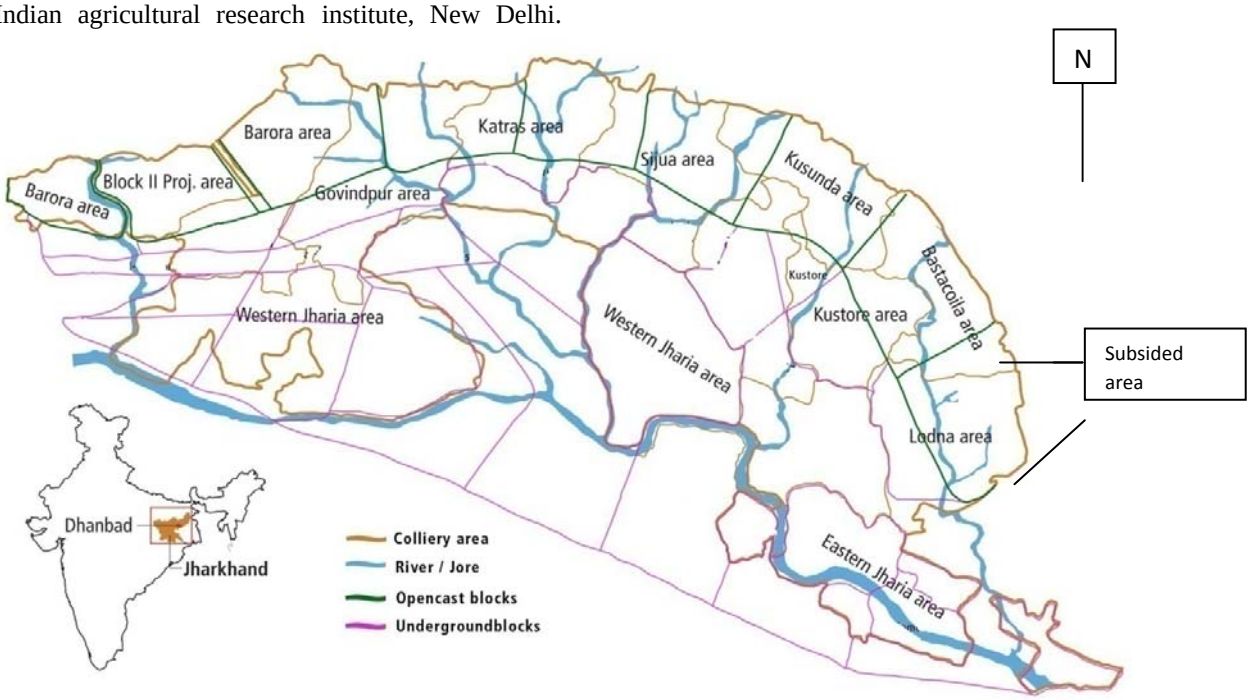

Figure 2. General map of study area.

2.4 Laboratory methods: The following methods are briefly mentioned underlined.

pH The pH value which is a measure of the hydrogen or hydroxyl ion activity of the soil water system indicates whether the soil is acidic, neutral or alkaline in reaction. Crop growth suffers much both under very low as well as high pH.

The instrument for pH measurement commonly used is a digital pH meters have single electrode assembly. The instrument being a potentiometer, the pH scale has to be calibrated before use with buffer solutions of known pH values. 20 gm of soil is taken in a 100ml beaker to which 40 ml of distilled water is added (Ghosh., et al 1983). The suspension is stirred at regular intervals for 30 minutes and the pH is recorded. The suspension must be stirred well just before the electrode are immersed and readings taken.

Electrical conductivity The conductivity of the supernatant liquid (after pH determination) is determined with the help of salt bridge.

Moisture content The standard method for determining moisture content of soil is the oven-drying method. This is the procedure recommended for soil. Moisture content measured by gravimetric method and expressed as percentage. Loss of weight of the samples was calculated to determine the moisture content.

Organic carbon The soil is grounded and completely passed through 0.2mm sieve (80mesh) and 1gm is placed at the bottom of a dry 500ml conical flask. Add 10 ml of potassium dichromate (1N) in the 500 ml conical flask, swirled and conical flask gently to disperse the soil in the dichromate solution. Then 20 ml of sulphuric acid is run in run in and swirled again two or three times. The flask is allowed to stand for 30 minutes and thereafter 200ml of distilled water along with 10 ml of ortho – phosphoric acid is added and 1ml of diphenylamine indicator. The whole contents are titrated with ferrous ammonium sulphate solution till the color flashes from blue – violet to green. For a final calculation, a blank is run without soil.

Available nitrogen In an 800 ml dry Kjeldhal flask, 20 gm of soil is taken. 20 ml of water, 100 ml potassium permanganate and 100 ml sodium hydroxide. The frothing during boiling is prevented by adding 1ml paraffin or a few glass beads. The whole contents are distilled in a Kjeldhal assembly at a steady rate and the liberated ammonia collected in a conical flask (250 ml) containing boric acid solution (with mixed indicator). With the absorption of ammonia the pinkish color turns to green. Nearly 100ml of distillate is to be collected in about 30 minutes which is titrated with (0.02N) sulphuric acid to the original pinkish color. For the final correction blank is run without soil (Subbiah and Asija. 1956).

Available phosphorus In a 25 ml volumetric flask, 5ml of the soil is taken and adding 5ml of dickman and bray reagent. The neck of the volumetric is washed down and the contents are diluted to about 22ml, then 1ml of dilute stannous chloride solution is added and volume is made up to the mark. The intensity of the blue color is measured (using 660nm) just after 10 minutes and the concentrations phosphorus is determined from the standard curve (Olsen, S. R, 1954).

Figure 3. Degradation of top soil

Table1.Top Soil Quality Sampling Stations within the Jharia coalfield**.**

S.No	Sampling sites	Latitude	Longitude
1	Kujama	23^0 44’ 15”	86^0 25’ 28”
2	Goluckdih	23^0 42’ 30”	$86^0$27’ 46”
3	North Tisra	23^0 42’ 35”	86^0 26’ 50”
4	South Tisra	23^0 43’ 06”	86^0 26’ 30”
5	Lodna	23^0 43’ 30”	86^0 25’ 55”
6	Jealagora	23^0 42’ 40”	86^0 25 ‘20”
7	Jeenagora	23^0 42’ 40”	86^0 26’ 48”
8	Joyrampur	23^0 42’ 45”	86^0 26’28”
9	Bagdigi	23^0 42’ 30”	86^0 25’ 39”
10	Bararee	23^0 42’ 12”	86^0 25’ 41”

3. Results & Discussions The results of the various soil quality parameters of JCF area are presented in Table 2.

Table 2. Top soil quality of various parameters of South Eastern part of JCF.

S.No	Sampling Locations	pH	Electrical Conductivity (mmhos/cm)	Moisture Content (%)	Organic Matter (%)	Available Nitrogen (kg/ha)	Available Phosphorus (kg/ha)
1.	Kujama	4.8	0.61	5.8	0.28	93.45	3.42

2.	Goluckdih	4.2	0.68	6.9	0.21	90.64	3.67
3.	North tisra	4.5	0.74	6.1	0.38	95.72	4.87
4.	South tisra	4.3	0.83	4.9	0.32	95.68	4.21
5.	Lodna	4.8	0.89	6.4	0.24	94.78	3.98
6.	Jealagora	5.7	0.82	7.1	0.28	97.21	4.86
7.	Jeenagora	5.2	0.72	6.9	0.31	110.65	4.62
8.	Joyrampur	5.6	0.69	5.9	0.33	107.82	5.38
9.	Bagdigi	5.3	0.76	7.2	0.41	98.64	5.51
10.	Bararee	5.8	0.73	7.5	0.39	98.94	4.97

From the above results it could be observed that the pH of soil in the subsided areas varied between 4.2 - 5.8.Top soil in subsided zone has slightly low pH. This may be due to oxidation of pyrite which is generally present in coal controls the lowering of pH. Low pH were responsible for most of the nutrients can be readily not available to plants. The total range of the normal soil pH scale is from 0-14. Values below the mid-point (pH 7.0) are acidic and those above pH 7.0 are alkaline. pH of the soil is the measure of hydrogen ion activity and depends largely on relative amounts of adsorbed hydrogen and metallic ions. The desired pH for good vegetation ranges from 5.5 to 6.8.pH is a good measure of acidity and alkalinity of soil water suspension and it provides a good identification of soil chemical nature (Sharma, H.P. 2008). `

Electrical conductivity (EC) of top soil varied between 0.61 mmhos/cm - 0.89 mmhos/cm. These values showed that conductivity of top soil increasing in subsided areas. The higher conductivity values in subsided zone are due to upward migration of salts along with them through cracks or fissures (Tripathy, et al., 1998). The EC value depends on the dilution of the soil suspension, total salts and sodium content. High value of EC can toxic to plants and may prevent them from obtaining water from the soil.

The observed moisture content was ranging from 4.9 % to 7.5% .The minimum and maximum values were recorded at South tisra and Bararee. The moisture content of subsided area is low as compared to normal area. The organic carbon content in top soil in degraded areas varied from between 0.21% -0.41%.The minimum and maximum values were recorded at Goluckdih and Bagdigi. It showed a decreasing of organic carbon from non degraded areas to degraded areas. Low organic content in subsided zones is because of burning out of organic matter present in top soil, low rate of humiliation and lack of microbes in top soil. The available nitrogen content fluctuated between 90.64 kg/ha to 110.65 kg/ha. The minimum and maximum value recorded at Goluckdih and Jeenagora respectively. Lower value of nitrogen in subsided zone is due to loss of organic carbon which contains nitrogen and nitrogen fixing microorganisms in soil.

The available phosphorus content fluctuated between 3.42 kg/ha to 5.51kg/ha. The minimum and maximum value recorded at Kujama and Bagdigi respectively. The value of phosphorus content is low to medium range in subsided zone. Low phosphorus in soil is because of its presence in insoluble state or due to lack of organic matter in soil.

4. Conclusions

From the present study,it can be concluded that the top soil in subsided zone has degraded slowly day by day. During mining operations, some steps must be taken, by good planning and environment

management to minimize effects of soil erosion, degradation, dust pollution and impacts on local biodiversity. Keeping above view in consideration for safe (less degraded), environmentally friendly and sustainable mine planning the most important part is the effective management of degraded land. Opencast mining should be planned in such a way that after the closure of the mine area it can be afforested to merge with the surrounding forest areas.

It is being suggested thus that all coal mining activities in Jharia coalfield, if conducted with a pre-defined land use management plan, to ensure that soil cover over the region will not be decreased, it will result in eco friendly mining.

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Potential Role of Arginine, Glutamine and Taurine in Ameliorating Osteoporotic Biomarkers in Ovariectomized Rats

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Abstract

The main purpose of the present study was to evaluate the role of some amino acids namely L-arginine, L-glutamine and taurine in the management of osteoporosis in ovariectomized (OVX) rats. The current study included six groups of female rats which were classified as gonad intact control group and five ovariectomized groups: one untreated group served as ovariectomized control group another ovariectomized group orally administered with 10% lactose, three ovariectomized groups orally administered with each amino acid dissolved in 10% lactose. The treatment was started after 3 months of ovariectomy and continued for other 3 months. Serum parathyroid hormone (PTH), 1, 25 dihydroxyvitamine D_3 levels were determined. Insulin like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β) levels were also estimated. Bone mineral density (BMD) and bone mineral content (BMC) of right femur bone of each rat were measured using DEXA technique. Also, histological investigation of the bone sections of left femur of each rat was carried out. The obtained data revealed that ovariectomy decreased serum 1, 25 $(OH)_2 D_3$, IGF-1 and TGF-β levels whereas, it increased serum PTH level. DEXA results revealed that ovariectomy decreased BMD and BMC of the proximal, distal and mid areas of rat femur bone. These results were well documented by bone histological examination. The selected amino acids could improve all the studied bone biochemical markers significantly. DEXA results also showed that treatment with these amino acids could increase both BMD and BMC of rat femur bone in most areas. The photomicrographs of femur bone sections of rats treated with the selected amino acids supported the present improvement in bone biomarkers. In conclusion, each of the selected amino acids exhibited antiosteoporotic effects due to the anabolic and/or antiresorptive activity. These encouraging results provide new concepts for the development of effective opportunities in the treatment of primary osteoporosis. "[Report and Opinion. 2009;1(6):24-35].(ISSN:1553-9873)".

Key words: Osteoporosis, L-arginine, L-glutamine, taurine, lactose, bone biomarkers, bone mineralization.

Introduction

Osteoporosis is a global health problem that will take an increasing significance as people live longer and the world's population continues to increase in number, thus the management of osteoporosis and its complications is an socioeconomic priority **(Kevin, 2007)** Osteoporosis is defined as decreased bone strength and increased susceptibility to fractures (**Preisinger , 2009)**. It is also defined as progressive systemic skeletal disease characterized by low bone mass with a consequent increase in bone fragility and susceptibility to fracture. **(Katherine et al., 2007).** So, there is an urgent need to develop and implement alternative nutritional approaches and policies for treatment of osteoporosis **(Kevin, 2007).** This idea comes from the fact that protein under nutrition is known to play an important role in the pathogenesis of osteoporotic fracture. The mechanisms underlying the bone loss in protein under nutrition appeared to be related to an uncoupling between increased bone resorption and bone formation. This was associated with decreased plasma insulin-like growth factor-1 (IGF-1) level, with anoestrus and decreased muscle mass. Nutritional intervention with amino acid supplements can increase bone mineral mass, bone strength and muscle mass in osteoporotic subjects **(Ammann et al., 2000).**

Amino acids are the building blocks of protein. Essential amino acids (EAA) can
Modulate the growth and the differentiation of osteoblasts cultured in vivo, confirming the relationship between osteoporotic hip fracture and inadequate protein intake. Amino acids have mainly enhanced cell growth and alkaline phosphatase activity, and, to a lower degree, collagen synthesis **(Conconi et al., 2001).**

Amino acids supplement increased bone mineral mass and strength in ovariectomized protein-deprived rats. This was associated with stimulated bone formation and reduced bone resorption, with an increment of plasma isulin-like growth factor (IGF-1) and limb muscle mass weight **(Ammann et al., 2000).**

L-arginine represents a key building block to repair damage tissue and bone. Athletes have also

found L-arginine to be beneficial for muscle recovery and growth hormone (GH) release from pituitary gland. Oral administration of L-arginine in pharmacological doses induces growth hormone and insulin –like growth factor-1 responses and stimulates nitric oxide synthesis **(Baecker et al., 2005)**. Growth hormone and insulin –like growth factor-1 is important mediators of bone turnover and osteoblastic bone formation, while nitric oxide is a potent inhibitor of osteoclastic bone resorption. Because of this dual effect on physiological regulators of bone remodeling, L-arginine could potentially increase bone formation over bone resorption, and consequently, increase bone mass **(Clementi et al., 2001)**.

Glutamine has a number of unique properties suggesting that this amino acid plays an important role in health and disease. This amino acid makes more than 60% of the skeletal muscle tissue, and it is a fuel for both the digestive tract and the immune system. Also, it is playing a pivotal role in conducting nitrogen to muscle around the body **(Tapiero et al., 2002).** Glutamine may at least in part play a role in mechanisms associated with cellular proliferation and/or differentiation through particular glutamine receptors (GluR) and glutamine transporters functionally expressed in rat calvarial osteoblasts **(Yoneda and Hinoi, 2003).** The cyclization of glutamate produces proline, an amino acid important for synthesis of collagen and connective tissue **(Tapiero, et al. 2002).** Therefore, it has been suggested that glutamine may have a role in the process of bone formation.

Significant amount of taurine is transported to bone tissue, it is reasonable to propose that taurine may play an important role in bone metabolism. Interestingly, taurine has been found to inhibit experimental bone resorption and osteoclast formation and survival **(Koide et al., 1999).** It has inhibitory effects on bacteria-stimulated osteoclast formation *in vitro*. Moreover, this amino acid has stimulatory actions on alkaline phosphatase activity and collagen synthesis **(Park et al., 2001).** It may play a role in osteoblastic differentiation as well as bone matrix formation. Taurine has anti-osteopenic effect in low Ca diet-induced osteopenia in rats, thereby promoting mineralization and finally leading to its bone anabolic action **(Yasutomi et al., 2002).**

Pharmacological mixture containing amino acids and lactose accelerates and ameliorates bone fracture healing processes. This finding is linked not only to calcium metabolism but also to different biological properties which positively contribute to good healing of bone fractures **(Fini et al., 1996).**

The principal goal of the current study was to develop alternative nutritional therapeutic modalities for the treatment of primary osteoporosis in order to avoid the serious side effects of the traditional hormone replacement therapy for osteoporosis in postmenopausal women. The suggested therapeutic opportunity included the supplementation of some promising amino acids The selected amino acids include L-arginine, L-glutamine, or taurine as effective dietary supplements for management of primary osteoporosis.

Materials and Methods:

Amino acids: L-arginine, L- glutamine, taurine and lactose were purchased from Sigma Company (U.S.A). **Experimental animals:** Adult female Sprague Dawley rats (120-150g) were obtained from Animal House Colony of the National Research Centre, Cairo, Egypt. The animals were kept in wire bottomed cage at room temperature (25± 2 ºC) under a 12h dark- light cycle and acclimated to the laboratory environment for seven days before use. Animals were fed with standard laboratory diet and water *ad libitum.* The rats were ovariectomized surgically in Hormone Department, Medical Research Division at the National Research Centre. Then, after three months following surgery, the animals were divided into 6 groups as follows: The first group; was untreated (OVX) rats and served as (OVX) control. The second group; (OVX) rats which were orally administered with (1ml/rat/day) lactose 10%. The third group; (OVX) rats which were orally administered with L-arginine dissolved in 10%lactose in a dose of 500mg/kg/day **(Gupta et al., 2005).** The fourth group; (OVX) rats which were orally administered with L-glutamine dissolved in 10% lactose in a dose of 3.2g/kg/day **(Ann et al., 2004).** The fifth group; (OVX) rats which were orally administered with taurine dissolved in 10% lactose in a dose of 50mg/kg/day **(Centiner et al., 2005)**. Additional untreated gonad intact control group was involved in the present study. The experiment lasted for 3 months.

At the end of the experimental period, the animals were kept fasting for 12 hours and the blood samples were collected from the retro-orbital venous plexus under diethyl ether anesthesia **(Schermer, 1967)**. The blood samples were left to clot and the serum were separated by cooling centrifugation (4º C) at 3000 rpm for 10 min. Serum parathyroid hormone (PTH) was estimated by ELISA procedure according to the method described by **Blum et al. (1993)**. Serum 1, 25-dihydroxyvitamin D_3 (Vitamin D_3) was determined by Radio immuno assay (^{125}I RIA) according to the method of **Hollis (1986).** Serum insulin-like growth factor-1 (IGF-1), transforming growth factor-β (TGF-β) were

determined using ELISA procedure according to the method of **Blum et al. (1993) and Kim et al. (1994)** respectively. The right femur bone of each animal was dissected, cleaned and stored in formalin buffer 10% for measuring bone mineral density (BMD) and bone mineral content (BMC) using dual energy X-ray absorptiometry (DEXA). The left femur bone was also carefully removed cleaned and stored in 10% formic acid solution as a decalcifying agent for 10 days for histological investigation.

Histological Examination:

The left femur was embedded in paraffin wax and the microscopic sections of 5µm intervals were taken and stained with hematosylin and eosin (H & E) for histological examinations (**Drury and Wallington, 1980**).

Statistical Analysis:

In the present study, all results were expressed as mean ± S.E of the mean. Data were analyzed by one way analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) program, version 11 followed by least significant difference (LSD) to compare significance between groups (**Armitage and Berry, 1987**). Difference was considered significant when P value ≤ 0.05.

Results:

1- Effect of Amino Acids Supplementation on PTH and Vitamin D:

Our data indicated that ovariectomy induced significant increase in serum PTH level associated with significant decrease in serum 1, 25 $(OH)_2D_3$ level in comparison with gonad intact control group. Treatment of ovariectomized rats with arginine, glutamine or taurine caused significant decrease in PTH serum level and significant increase in 1, 25 $(OH)_2D_3$ serum level comparing with ovariectomized rats that administered lactose only (Table 1).

Table (1): Effect of Different Amino Acids Supplementation on Serum Parathyroid Hormone (PTH) and 1,25 $(OH)_2D_3$ Levels in Ovariectomized Rats.

Parameters / Groups	PTH Pg/ml	1,25 $(OH)_2D_3$ (Pg/ml)
Gonad intact control	36.6±2.8	15.7±0.3
OVX Control	62.5±2.4 [a]	12.8±0.2 [a]
OVX + Lactose	60.4±2.1	13.2±0.25
OVX + Arg.	50.2±2.4 [b]	15.3±0.34 [b]
OVX + Glut.	49.4±1.7 [b]	15.2±0.18 [b]
OVX + Tau.	45.9±2.8 [b]	15.7±0.14 [b]

a : Significant change at P 0.05 in comparison with gonad intact control.
b : Significant change at P 0.05 in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats, Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Table (2): Effect of Different Amino Acids Supplementation on Serum Insulin-like Growth Factor-1 (IGF-1), and Transforming Growth Factor-β (TGF-β) Levels in Ovariectomized Rats.

Parameters / Groups	IGF-1 Ng/ml	TGF-β Pg/ml
Gonad intact control	9.7±0.7	193.9±9.1
OVX Control	6.7±0.32 [a]	125.8±2.7 [a]
OVX + Lactose	7.0±0.19	131.1±2.3
OVX + Arg.	8.2±0.21 [b]	160±4.0 [b]
OVX + Glut.	8.5±0.16 [b]	171.9±2.7 [b]
OVX + Tau.	8.8±0.22 [b]	179.8±2.1 [b]

a : Significant change at P 0.05 in comparison with gonad intact control.
b : Significant change at P 0.05 in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

2- Effect of Amino Acids Supplementation on IGF-1 and TGF-β:

The present data revealed that ovariectomy resulted in significant decrease in each of IGF-1 and TGF-β in serum as compared to gonad intact control group. Treatment with the tested amino acids produced significant increase in IGF-1 and TGF-β serum levels in comparison with ovariectomized rats received lactose only as represented in Table (2).

3- Effect of Amino Acids Supplementation on BMD and BMC:

The results in table (3) showed that ovariectomy decreased BMD of proximal, mid and distal areas significantly in comparison with gonad intact control group. While ovariectomized rats treated with arginine showed significant increase in BMD of proximal and mid areas and insignificant increase in BMD of distal area in comparison with ovariectomized rats received lactose only. Treatment with glutamine or taurine induced significant increase in BMD of proximal, mid and distal areas in comparison with ovariectomized rats received lactose only. Noteworthy, ovariectomized rats treated with taurine produced significant increase in BMD of proximal, mid and distal areas in comparison with untreated ovariectomized group.

Table (3): Effect of Different Amino Acids Supplementation on Bone Mineral Density (BMD) in Ovariectomized Rats.

BMD (mg/cm^2)			
Parameters / **Groups**	Proximal mg/cm^2	Mid mg/cm^2	Distal mg/cm^2
Gonad intact control	126.4±2.5	126.2±1.9	131.0± 1.5
OVX Control	109.4±1.4 [a]	112.8±2.2 [a]	115.2± 2.9 [a]
OVX + Lactose	110.7±1.5	114.3±2.2 [a]	117.0± 2.1
OVX+ Arg.	117.2±1.9 [b]	117.6±0.7 [b]	122.6± 1.0
OVX+ Glut.	119.3±1.8 [b]	119.8±1.0 [b]	125.7± 1.0 [b]
OVX +Tau.	120.7±1.4 [b]	120.6±1.4 [b]	127.2± 1.9 [b]

a: Significant change at $P \leq 0.05$ in comparison with gonad intact control.
b : Significant change at $P \leq 0.05$ in comparison with ovariectomized received lactose group.
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Our data in table (4) indicated that ovariectomy induced significant decrease in BMC of proximal and distal areas and insignificant decrease in BMC of mid area in comparison with gonad intact control group. Treatment with arginine, glutamine or taurine caused significant increase in BMC of proximal area comparing with ovariectomized received lactose only. While amino acids supplementation induced insignificant increase in BMC of mid area as compared to ovariectomized rats received lactose only with respect to the value of BMC of distal area only, glutamine and taurine supplementation showed significant increase, while supplementation with arginine caused insignificant increase as compared to ovariectomized rats received lactose only. It could be also seen that taurine supplementation increased BMC significantly in comparison with supplementation with arginine.

Table (4): Effect of Different Amino Acids Supplementation on Bone Mineral Content (BMC) in Ovariectomized Rats.

BMC (mg/cm^2)			
Parameters / **Groups**	Proximal	Mid	Distal
Gonad intact control	72.9±1.0	208.3±7.0	77.6±1.7
OVX. control	44.9±1.5 [a]	182±4.0	50.6±2.1 [a]
OVX + Lactose	45.4±1.0	186.8±3.2	55.8±3.3
OVX + Arg.	56.7±3.5 [b]	191.3±3.7	64.1±0.9
OVX + Glut.	59.2±3.0 [b]	195.5±2.1	67.0±0.8 [b]
OVX + Tau.	65.2±1.2 [b]	199.2±4.3	70.3±2.0 [b]

a : Significant change at $P \leq 0.05$ in comparison with gonad intact control.
b : Significant change at $P \leq 0.05$ in comparison with ovariectomized received lactose group
OVX.: Ovariectomized rats. Arg.: Arginine, Glu.: Glutamine, Tau.: Taurine

Histological Results:

Microscopic examination of left femur bone section of gonad intact control rat represented in **Fig. (1)** showed a network of bony trabiculae separated by a labyrinth of interconnecting spaces containing bone marrow. The trabiculae composed of irregular lamellae of bone with Haversian systems and lacunae containing osteocytes.

The photomicrograph of left femur bone section of untreated ovariectomized control rat in **Fig. (2)** showed the reduction of the cortical and trabicular bone thickness. Many of necrotic areas of bone and resorped cavities on the inner surface have been also seen.

Micrograph of a longitudinal section of left femur bone of ovariecomized rat received lactose **(Fig. 3)** showed some necrotic areas of bone and the presence of small cavities.

The photomicrograph of left femur section of rats treated with arginine showed the new formed bone and increase in the thickness of bone **(Fig. 4)**.

It is clear from the photomicrographs of left femur bone sections of ovariectomized rats treated with glutamine, the presence of calcified cartilage and the increased thickness of the bony trabiculae (**Fig. 5**).

A section of left femur bone of ovariectomized rat treated with taurine showed the trabiculae appeared as normal form **(Fig. 6)**.

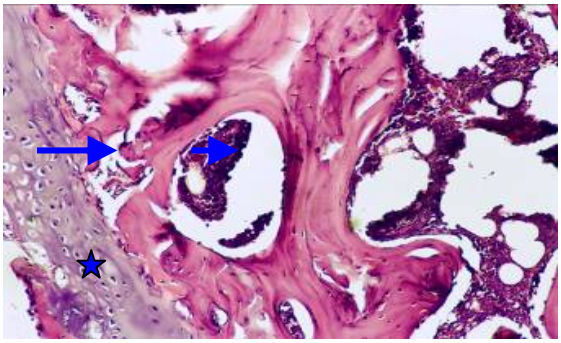

Figure (1): Photomicrograph of femur bone section of gonad intact control rat showing a network of bony trabiculae separated by a labyrinth of interconnecting spaces containing bone marrow. The trabiculae composed of irregular lamellae of bone with Haversian systems and lacunae containing osteocytes (H & E X 400).

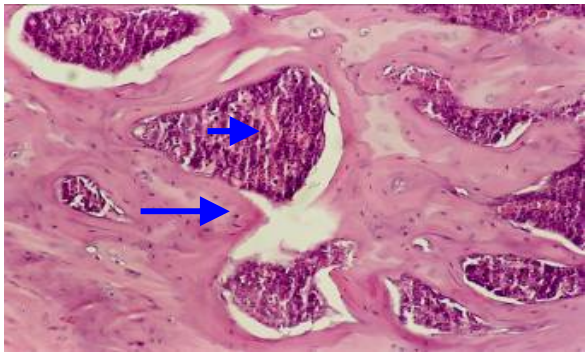

Figure (2): Micrograph of a longitudinal section of left femur bone of ovariecomized rat showing the reduction of the cortical and trabicular bone thickness. Many of necrotic areas of bone and resorped cavities on the inner surface are also seen. Cartilage layer in the trabecular bone is found (H&E x 150).

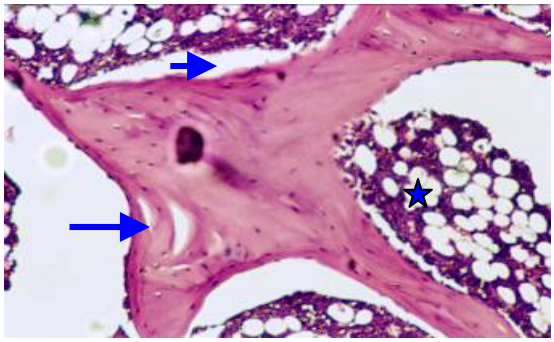

Figure (3): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with lactose showing some necrotic areas bone and the presence of small cavities. The erosion of the outer surface of bone is also appeared (H & E X 150).

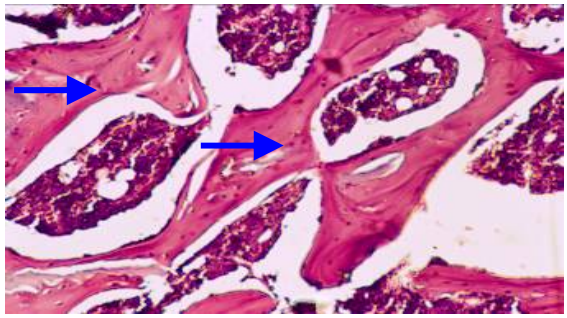

Figure (4): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with arginine in lactose showing an increase in the thickness of bone (H & E X 150).

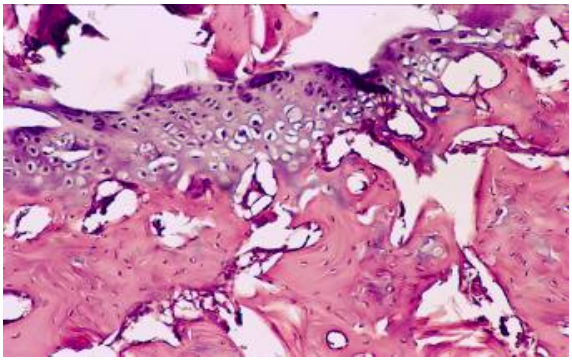

Figure (5): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with glutamine in lactose showing the increase in the number of trabiculae (H & E X 150).

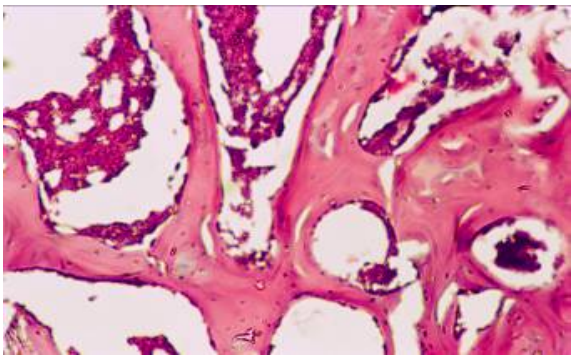

Figure (6): Micrograph of a longitudinal section of left femur bone of ovariecomized rat treated with tuarine showing normal appearance of bone (H & E X 150).

Discussion

Osteoporosis is a chronic condition chiefly affecting postmenopausal women, in whom the skeleton loses a significant percentage of its mineralized mass and mechanical resiliency, thereby becoming prone to fracture (**Fan et al., 2005).** Amino acids have been shown to stimulate bone formation and thus, they might be represented useful agents for the prevention and treatment of osteoporosis **(Conconi et al., 2001).**

The present study showed that ovarietomy induced significant decrease in all the tested parameters except PTH which increased significantly comparing with gonad intact control group. These findings are in agreement with **Segal et al, (2003) and Chen et al, (2007).** It could be explained that ovariectomy induced increase in PTH gene expression and parathyroid cell proliferation(**Silver et al., 1999).**The decrease in 1,25 $(OH)_2D_3$ serum level in ovariectomized animals may be due to that estrogen loss is the reason of reducing $1,25(OH)_2D_3$ production and calcium absorption in this rat model **(Ash and Goldin,1988),** While, the reduction of serum IGF-1 level may be explained as estrogen stimulates the autocrine secretions of IGF-1 and TGF-β by osteoblasts which are involved in the stimulation of osteoblasts maturation and growth as well as collagen synthesis and alkaline phosphatase secretion (**Kajdaniuk et al., 1999).** Many reports indicated that reduced plasma levels of IGF-1 are associated with estrogen deficiency and in turn osteoporosis in females. **Calo et al, (2000)** reported that ovariectomy resulted in significant reduction in the number of receptors for both epidermal growth factor (EGF) and IGF-1 in female rats. On the other hand, the reduction in TGF-β resulted from a direct effect of estrogen deficiency on bone cells to decrease the secretion of TGF-β with a concomitant decrease in the deposition of newly formed bone (**Finkelman et al., 1992**).. This possibility was further supported by the findings that 17β-estradiol administration directly stimulated the production of TGF-β by mouse bone cells and also estrogen replacement therapy *in vivo* corrected the TGF-β deficit (**Finkelman et al., 1992**). Moreover, it has been reported that estrogen deficiency led to a decrease in TGF-β mRNA which may simply be due to the reduction in cancellous bone volume that occurs following ovariectomy **(Westerlind et al., 1994).**

The current study also showed a reduction in BMC and BMD in ovariectomized animals as compared to the gonad intact control animals. These results may attribute to that estrogen deficiency resulted in rapid bone loss phase which is associated with loss of BMD **(Shen et al., 2000).** It has been reported that the reduced BMD in ovariectomized rats is also associated with a reduction in dry and ash femur weights as well as a decreased femoral breaking force and energy suggesting the increased risk of fracture **(Park et al., 2008).** The mechanism by which estrogen deficiency could induce bone resorption is that loss of estrogen induced enhanced expression of bone resorbing cytokines (interleukin-1 (IL-1), tumor necrosis factor-alpha (TNF-α) **(Kitazawa et al., 1994)**, interleukin-6 (IL-6) **(Passeri et al., 1993)** and macrophage colony stimulating factor (M-CSF) **(Kimble et al., 1996)** by immune cells and osteoblasts in the bone marrow microenvironment **(Matsushita et al., 2008).** These cytokines are crucial for the pathogenetic mechanisms by which estrogen deficiency leads to increase the expression of functional receptor activator nuclear factor kappa B ligand (RANKL) and to enhance bone resorption and bone loss **(Kwan Tat et al., 2004),** via increasing osteoclast number and activity **(Kassam, 2003)** and promotion nuclear

factor kappa B, the key transcription factor in osteoclastogenesis **(Ross, 2003).** Ovariectomy-induced bone resorption could be responsible for the decreasing in calcium and mineral content of the whole femur **(Gaumet et al., 1996).**

Our study revealed that lactose administration in ovariectomized rats led to slight inhibition in serum PTH and slight increase in all of the other tested parameters. These results could be explained as lactose slightly increase serum level of Ca and ionized Ca which inhibit the secretion of PTH, leading to increasing the circulating level of 1,25 $(OH)_2$ D_3 by stimulating calcitriol synthesis **(Mastaglia et al., 2006).** Moreover the increase in serum IGF-1 and TGF-B levels with lactose administration may be explained as lactose has a stimulatory effect of lactose on osteoblasts growth and the production of several growth factors **(Kirk et al., 1994).**

It has been found that lactose supplementation to ovariectomised rats induced increase in BMD and BMC, which could be explained as dietary lactose increased bone calcification rate and inhibited bone resorption that lead to the improvement in skeletal growth and mineralization in animals fed lactose **(Shortt and Flynn,1991).**

Our data revealed that arginine supplementation to ovariectomized rats induced significant decrease in PTH level while, it showed significant increase in1,25$(OH)_2D_3$,IGF-1,TGF-β and both of BMD and BMC when compared with the ovariectomized rats administered lactose only. Arginine via growth hormone has been found to produce marked increase in (1, 25$(OH)_2$ D_3) due to increasing nephrogonous cyclic AMP(N_cAMP) **(Ahmad et al., 2003)**. The increase in IGF-1 level may be due to the role of arginine in stimulating IGF-1 production and collagen synthesis in osteoblasts-like cells. It has been suggested that arginine could increase IGF-1 mRNA transcription and alpha (1) collagen mRNA transcripts and thus, arginine may influence bone formation by enhancing IGF-1 production **(Chevalley et al.,1998**). Lactose appeared to have an intangable role in enhancing serum level of IGF-1 in ovariectomized rats via stimulation of osteoblasts cells growth, but the major role in this respect could be attributed to arginine supplementation. Also arginine directly increased the expression of TGF-β mRNA and TGF-β protein levels as it could increase the production and deposition of matrix components **(Narita et al., 1995).** Furthermore the increase in BMD due to arginine supplementation could be explained as arginine has a dual effect on physiological regulators of bone remodeling. Arginine could potentially increase bone formation over bone resorption, and consequently, increase bone mass **(Van't Hof and Ralston, 2001).** Additionally, there is growing evidence demonstrated that moderate concentrations of nitric oxide (NO) play an essential physiological role in promoting maintenance of bone density-stimulating new bone formation while suppressing bone catabolism **(Armour et al., 2001).**

Our data indicated that glutamine administration to ovariectomized rats tends to significantly increase 1,25$(OH)_2D_3$,IGF-1,TGF-β,BMC and BMD. While it could significantly decrease serum PTH level when compared to ovareiectomized rats administered lactose only.

It has been reported that glutamine supplementation produces glutathione which acts as a potent enhancer of calcium through activation of calcium sensing receptor (CaSR). Glutathione acts as an endogenous modulator of this receptor particularly in the parathyroid gland where this receptor is known to control parathyroid hormone release **(Wang et al., 2006).** Also glutathione could increase circulating 1,25 $(OH)_2D_3$ via stimulating enzymatic activity of the renal 1 alpha-hydroxylase of 25-hydroxycholecalciferol **(Schedl et al.,1992).** Furthermore glutamine supplementation increases IGF-1 level due to its conversion to alpha-ketoglutarate (αKG) in the body. Alpha - ketoglutarate has been shown to increase circulating plasma levels of insulin, growth hormone with consequent increase in IGF-1 **(Jeevanandam and Petersen, 1999)**. Growth hormone could stimulate osteoblastic proliferation and differentiation and increase the production of IGF-1 **(Corpas et al., 1993).**

The role of glutamine in inducing the detectable increase in BMD of right femur areas of ovariectomized rats mainly depends on its anabolic effect on bone, since glutamine played specific role in mechanisms associated with cellular proliferation and/or differentiation through particular receptors and transporters functionally expressed in rat calverial osteoblasts **(Yoneda and Hinoi, 2003).** Moreover, Alpha ketoglutrate has been found to increase mineralization, higher volumetric cortical bone density and increase trabecular bone density in animals **(Tatara et al., 2005).** Also alpha ketoglutrate is a component of antioxidant glutathione and polyglutamated folic acid. These antioxidants play a role in inhibiting osteoclastogensis via inhibition of reactive oxygen species (ROS) which are necessary for osteoclast activity and bone resorption **(Key et al., 1994)**. The positive effect of α-KG on bone was previously reported in birds since it could increase bone weight, mean relative wall thickness, maximum elastic strength, ultimate strength and volumetric bone

mineral density in birds **(Tatara et al., 2005)** Similar findings have been also reported in ovariectomized rats **(Radzki et al., 2002).** Finally, the cyclization of glutamine produces proline, an amino acid important for synthesis of collagen and connective tissue **(Tapiero et al., 2002)** which contributes to the positive influence of glutamine on bone tissue.

The suggested mode of action of α-KG on bone mineralization could be attributed to the efficacy of α-KG to maintain a delicate balance between bone resorption and bone formation that plays an important role in determining bone strength and integrity **(Rodan and Mertin, 2000).** Glutamine may play a role as a signal mediator in mechanisms associated with chondral mineralization through the group III m glutamine receptor (mGluR) subtype functionally expressed by chondrocytes in cartilage **(Wang et al., 2006).** Thus, glutamine could produce the increase in BMC of each of proximal, mid and distal areas of ovariectomized rat right femur bone through its indirect effect on mechanical properties of bone. Lactose has a significant role in promoting the effect of glutamine on BMC of the three regions of right femur bone of OVX rats in the present study.

The current results showed that taurine administration induced significant decrease in serum PTH level while it produced significant increase in each of $1,25(OH)_2D_3$, IGF-1,TGF-β,BMC and BMD in ovariecomized rats. The effect of taurine in decreasing PTH level could be attributed to its role in increasing magnesium concentration through the activation of extracellular signal regulated protein kinase (ERK) pathway **(Jeon et al., 2007).** The resulting elevation in magnesium concentration could suppress PTH secretion as magnesium positively affected intestinal calcium absorption and bone metabolism in ovariectomized rats **(Toba et al., 2000).** Taurine has been shown to have direct effect on accelerating vitamin D absorption and in turn increasing serum $1, 25(OH)_2 D_3$ level in ovariectomized rats treated with taurine. This suggestion is greatly supported by **Petrosian and Haroutounian, (2000). Gaylord et al. (2007)** stated that taurine stimulated pituitary growth hormone with subsequent stimulation of growth hormone – dependent IGF-1 in animals. Thus, growth hormone responsive and IGF-1 secreting cells might require sufficient taurine to secrete IGF-1 at normal levels **(Hu et al., 2000).** Taurine could produce the detectable increase in serum TGF-β level by two mechanisms, stimulatory action of taurine on osteoblastic differentiatation as well as bone matrix formation **(Park et al., 2001),** and indirect effect of taurine in increasing circulating level of $1,25 (OH)_2 D_3$ which in turn led to increasing TGF-β release from bone cells. Lactose may have a role in enhancing TGF-β level in contribution with taurine through stimulation of osteoblast growth and production of growth factors mainly TGF-β **(Petrosian and Haroutounian, 2000).** Moreover taurine has been found to promote osteoblasts mineralization and it could regulate osteoblasts metabolism via stimulation of extracellular signal regulated protein kinase phosphorylation **(Park et al., 2001).** Taurine might also have antiresorptive action through its antioxidant effect **(Lourenco and Camilo, 2002).** Therefore, we could suggest that taurine via scavenging reactive oxygen species, necessary for osteoclast function, could inhibit bone resorption. Therefore, through these common pathways, taurine has a preventive effect on bone loss. The unique role of taurine in modulating mitochondrial Ca^{2+} homeostasis might be of particular importance under pathological conditions **(Palmi et al., 1999).** Lactose may have a synergistic effect with taurine on increasing BMC of proximal, mid and distal regions of right femur bone.

Histological investigation of bone tissue sections showed that estrogen deficiency is associated with elevated bone resorption caused by a rise of osteoclast number**.** Recent study of **Park et al. (2008)** observed that the ovariectomized rats that exhibited osteoporosis within 7 weeks after surgery showed large decreases in the bone volume ratio and trabecular bone thickness.

Treatment with lactose showed some necrotic areas and appearance of small cavities in the bone. The ability of lactose to facilitate the passage of calcium across the intestine, resulting in improved calcium availability to the skeleton **(Miller et al., 1988). Marie and Travers (1983)** observed a slight decrease in osteocytes thickness in rats fed diet containing lactose.

Supplementation of ovariectomized rats with arginine revealed the formation of new bone. This finding could be explained in the view of the effect of arginine via growth hormone stimulation as well as IGF-1 production. Growth hormone has been found to have a positive effect on chondrocytes and osteoblasts **(Saggese et al., 1995)** as well as it could increase the number and function of osteoblasts **(Bouillon, 1991).** This is because of osteoblasts express functional growth hormone receptors (GHR) **(Nilsson, et al., 1995)** indicating that growth hormone (GH) also exerts a direct effect on osteoblasts. A direct effect of GH on osteoblasts is supported by earlier results for the epiphysial growth plate, where it has been demonstrated that GH interacts directly with epiphysial chondrocytes for the regulation of longitudinal bone growth **(Ohlsson et al., 1992)**.IGF-1 has been found to play a role in trabecular and cortical bone formation **(Conconi et**

al., 2001) IGF-1 showed a positive effect on bone formation in vitro as it could stimulate the formation of osteocalcin, collagen and non-collagenous matrix proteins by differentiated osteoblasts and increased the number of functional osteoblasts by promoting osteoprogenitor cell replication **(Visser and Hoekman 1994)**. Lactose assisted arginine in mainting plenty of calcium for strengthening the fragile bone, and increasing bone thickness as appeared in the current study.

Supplementation of ovariectomized rats with glutamine showed the calcification of cartilage, increased trabecular bone thickness and formation of new bone. This result could be attributed to the functional role of glutamine in chondral mineralization through the group III mGluR subtype functionally expressed by chondrocytes in cartilage **(Wang et al., 2006).** Moreover, glutamine via producing α-KG in the body could promoting bone weight, wall thickness and bone strength **(Tatara et al., 2005).** Recent study of **Polat et al. (2007)** demonstrated that glutamine had positive effects on healing of traumatically fractured bone through attainment of positive nitrogen balance. The role of lactose here is to improve calcium availability to facilitate the formation of new bone.

Taurine could restore the normal appearance of trabiculae as shown in the micrograph of bone tissue section of left femur of ovariectomized rats treated with taurine in the current work. Considering that a significant amount of taurine is transported to bone tissues, the transcription and translation of taurine occurs in bone forming cells **(Yuan et al., 2006)**. Therefore, it is reasonable to propose that taurine may play an important role in bone metabolism. Taurine in the osteoblasts activates extracellular signal regulated protein kinase-2 (ERK2) and phosphorylates transcription factors thus activating collagen gene transcription and protein synthesis. These actions of taurine may be beneficial for osteoblastic differentiation and bone matrix formation (**Park et al., 2001**). Additionally, taurine could stabilize cell membranes, eliminate oxide free radicals, regulate intracellular osmosis and maintain intracellular calcium concentration (**Pasantes-Morales et al., 1998**). It is clear that lactose could enhance the effect of taurine on bone formation through improving the organization of the trabecular bone in ovariectomized rats as shown in the present study from the normal appearance of bone following administration of taurine in lactose.

In conclusion, the present study provided clear evidence that ovariectomy produced marked abnormalities in bone biomarkers and in increasing risk of fracture. Also ovariectomy reduced plasma IGF-1 level and decreased TGF-β, as well as accelerated bone loss phase. The selected amino acids showed positive effect on bone via inhibiting the secretion of parathyroid hormone in concomitant with increasing serum 1,25 dihydroxyvitamin D_3 Also, the studied amino acids stimulated the production of IGF-1 and TGF-β and increased bone calcification rate as well as improved calcium availability to the skeleton. Lactose participated in enhancing the positive effect of the selected amino acids on bone. Arginine provided promising effect on bone through stimulation of insulin- like growth factor. Also, arginine via nitric oxide which is involved in increasing basal calcium absorption in small intestine thus stimulates the replication of primary osteoblasts and as well as inhibiting osteoclasting bone resorption. Glutamine through the production of alpha-ketoglutarate and glutathione showed potent effect on bone. These metabolic products of glutamine have a critical role in increasing bone density and strength in addition to bone mineralization. Taurine revealed the most effective action on bone remodeling via stimulating osteoblastic differentiation as well as bone matrix formation. In addition to taurine anabolic effect, it has an antiresorptive action through its antioxidant activity which participates in inhibiting osteoclast function and consequently bone resorption. Our finding might be useful for the future strategies against menopausal bone turnover and implicitly osteoporosis progression.

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The Genotoxic Effect of Sewage Effluent on *Allium Cepa*

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ABSTRACT: The genotoxicity of sewage effluent was investigated using both morphological and root chromosome assay. The mean root lengths of onions exposed to different concentrations of the effluent were measured every day for 5 days and EC_{50} values were determined from the growth curve as 47%.The result the mean root length was statistically evaluated by the analysis of variance and least significant difference. There was a significant decrease in root length of the experiment. Also the mitotic index decreased as concentration increased. Total aberrations increased significantly as concentration increased ($p<0.05$). These results demonstrate that the *Allium* test is a useful screening test for the evaluation of toxicity in sewage effluent. [Report and Opinion. 2009;1(6):36-41]. (ISSN: 1553-9873)

Key words: sewage, effluent, onions, genotoxicity, genetic damage.

INTRODUCTION

Sewage water has been used to support agricultural production in many countries over a considerable period of time. Grisolia *et al*, (2005), reported that in Basilia, capital of Brazil, sludge from sewage plant is recovered to be used to improve the organic content of agricultural soils. In Egypt, there are many examples of planned reuse of sewage effluents for irrigation of crops (El-Bagouri, 1999, Amin, 2002).

Effluent reuse can provide considerable benefit when used under controlled conditions to establish protection of health of both farm workers and consumers of the produce (Aleem and Malik, 2003).

The main components of sewage effluent that warrant consideration for irrigation include major and minor nutrients like nitrogen, potassium, phosphorous, calcium, iron, zinc, copper and manganese as well as heavy metals such as cadmium, lead, chromium and Mercury. There are also the presence of certain cations and anions which might cause adverse effect on soil properties and plant growth (Brown *et al.*, 1991, Amin and Migahid, 2000).

Friskesjo (1987) noted that with increasing interest in the use of cytological investigation in short term test (STIs) for environmental monitoring, higher plants have come into focus reviewing plant toxicity test. Wang (1992) has emphasised the sensitivity of simple root elongation test and recommends plants especially for toxicity testing for complex mixtures.

Several plant assay system have been used to monitor genotoxic substances in the environment. Bansal (1998) studied the impact of long term application of sewage on soil properties and residual affect of heavy metals uptake by barley and corn. The result revealed higher concentration of heavy metals when compared to fields irrigated with tube well water. Odeigah *et al*, (1997a) and (1997b) used the *Allium* test to evaluate the genotoxic effect of waste water and leachates from solid industrial wastes respectively. Their result showed that the contaminants have both macroscopic and microscopic effect on plants. However increased growth of olive trees and fodder grasses was observed for irrigation using sewage effluent (Saavedera *et al.*, 1984)

In Lagos State Nigeria, Sewage sludge is being used to cultivate vegetable crops like spinach, cabbage, carrot along Lagos- Badagry expressway. The choice of sewage sludge could be due to high cost of artificial fertilizer. An investigation has revealed that this sewage sludge were product of primary sedimentation (personal communication) thereby raising concern over the environmental implication of this practice.

The principal objective of this study is to assess the effect of untreated sewage water collected at Mile 2 dump site on the genetic material of *Allium cepa*. It is hoped that the result of this study may provide more information on the possibility of the use of treated waste water for irrigation farming.

MATERIALS AND METHODS

SEWAGE EFFLUENT:

The sewage effluent was obtained at mile 2 dump site in Lagos state, Nigeria. The samples were collected before their discharge into the coastal waters.

TEST MATERIAL

Onions bulbs were purchased at Tejuosho Market, Lagos. They were sun dried before use.

TEST PROCEDURE:

Planting of Onion

The outer scales of the bulbs and the brownish bottom plate were first removed. The rings of the root primordial were left intact. A series of cleaned small sized bulbs of onions, (*Allium cepa*) were first sprouted in water as described by Friskesjo (1987). After 24 hours, the best in terms of root growth were selected. Four each were placed on top of containers filled with 20%, 40%, 60%, 80% and undiluted aliquots of sewage effluent and fresh tap water for control and was termed as day one. The solutions were changed everyday during the experiment.

Root Length Measurement

The root length of onion bulbs from each concentration was measured on day 2, 3, 4 and 5 of the experiment using a calibrated ruler. The mean root length of each treatment in each concentration was calculated by dividing the total root length for each concentration by four. The root length of the control was also calculated and the result plotted on a graph.

Cytological Study:

The emerged root tips of the onion bulbs in the different concentration of sewage effluent were fixed in aceto-alcohol (1:3) after the second and fourth days of starting the experiment. The conventional feulgen-squash method (Sharma and Dphil, 1980) was used to prepare permanent slides of root meristems.

The root tips were put in 1 normal hydrochloric acid for five minutes to soften the tissue. The tips were them macerated and stained with aceto-orcein stain for 15 minutes. The macerated and stained root tips were covered with cover slip and squashed and later viewed in a microscope. The mitotic activity, rate and kind of aberrations were recorded using four replicates for each treatment.

The results of the mean root length were statistically evaluated by the analysis of variance and least significant difference (LSD) test at 5% significant level.

RESULT

MACROSCOPIC EFFECT

It was observed that sewage effluent suppressed root growth when compared with the control. At 100% concentration there was total growth inhibition and the roots decayed after 48 hours of the experiment while at 80% concentration the roots decayed after 72 hours. At 60% concentration their appeared crochet hooks and swellings at the root tips Signs of wilting started to appear on few of the treated roots after 48 hours especially at 80% concentration. The EC_{50} occurred at 47% (Figure 1).

MICROSCOPIC EFFECT:

Treatment with sewage effluent for 4 days lowered the mitotic activity significantly than the control. The mitotic index decreased with increasing the concentration of sewage effluent (Table 1). At 80% concentration and undiluted aliquot there was no mitosis. The aberration rate of non-dividing cells increased significantly as the concentration increased. ($p<0.05$) More than one kind of aberration was found per cell. The higher value was recorded in 20% concentration. Sticky metaphase chromosomes were the most frequent kind of aberration induced by sewage effluent. C-mitosis was only found in 20% concentration.

Abnormal chromosome orientation and movement such as multipolar anaphase and binucleus were found in 20% and 40% concentration (Plate 1). Dividing cells was highest in control experiment and decreased significantly as concentration increased (Table 1).

Table 1. Effects of treatment with different concentration of sewage effluent

	PHENOTYPIC INDICES				CHROMOSOME ABERRATIONS			
Concentration (%)	Root length % control	Mitotic index ±SD	Number of cells	Dividing cells	Stickiness	C-mitosis	Vagrant	Bridges fragments
Control	100	6.6 ± 0.46	500	33	8	0	0	0
20	97.5 + 5.8	3.9 ± 0.11	451	18	8	1	4	3
40	57.0+ 5.3	3.0 ± 0.10	396	12	7	0	3	5
60	29.1 + 3.2	2.9 ± 0.07	384	11	9	0	4	0
80	13.2 + 3.0	0	0	0	0	0	0	0
100	0	0	0	0	0	0	0	0

Mitotic index was calculated as mitotic cells per 1000 cells per slide;
Chromosomal aberrations were scored on 500 cells per slide

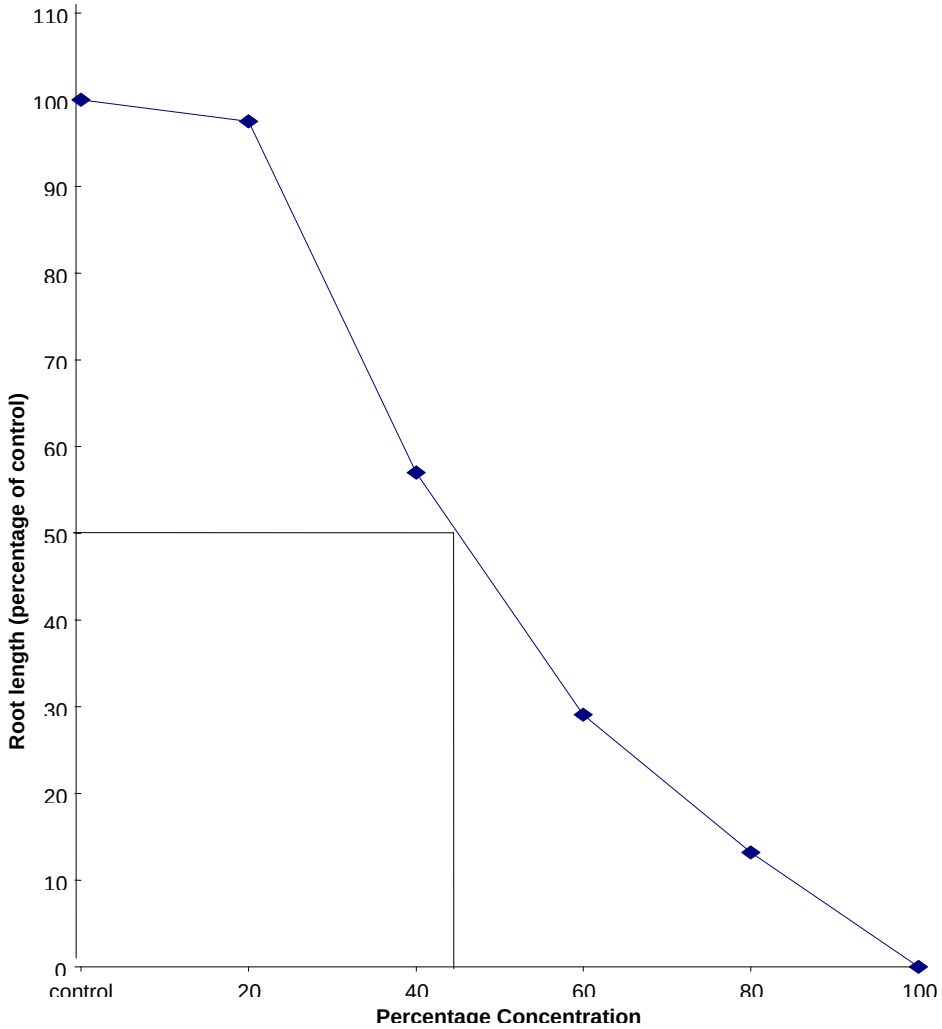


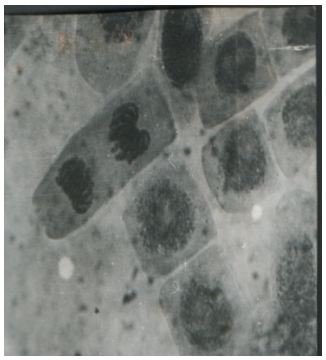
Fig. 1: Growth curve of Allium roots (in relations to control) after treatment with sewage effluents


Multipolar Telophase

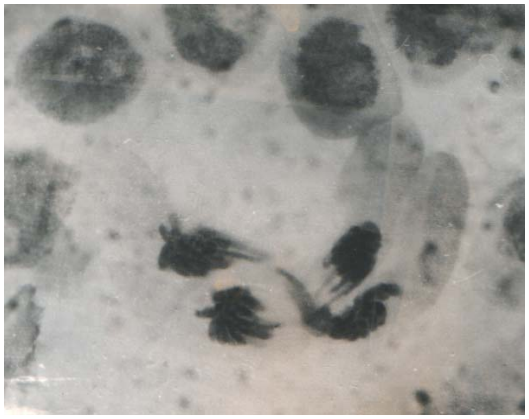

Binucleus


Vagrant and Multipolar Anaphase


Telophase with laggards


Sticky Telophase


Bridged and Fragmented Anaphase


Multipolar Anaphase


Vagrant Metaphase

Plate 1: Common chromosomal aberrations induced by sewage effluent.

DISCUSSION

Parameters such as root shape and growth, frequencies of mitosis and abnormal cell division can be used to estimate the cytotoxicity, genotoxicity and mutagenicity of environmental pollutant (Amin, 2002; Nielson and Rank, 1994). The *Allium* test has many advantages as genotoxicity screening assay, one being that *Allium* root cells posses the mixed function oxidase system which is capable of activating promutagens or genotoxic chemicals (Odeigah *et al*; 1997a). In the *Allium* test, inhibition of rooting and the appearance of stunted roots indicate retardation of growth and cytotoxicty, while root wilting explains toxicity (Odeigah *et al*; 1997b, Grant, 1982). Nevertheless both growth retardation and root wilting are accompanied by suppression of mitotic activity and occurrence of chromosomal aberration.

The present study provides evidence that sewage effluent inhibited root growth and caused growth retardation. The inhibition of growth may be due to high rate of chemical oxygen demand which affected certain physiological processes leading to the disturbance in the balance between promoter and inhibitors of endogenous growth regulator (Grover and Tejpar, 1981).

Growth inhibition was most marked at 100% concentration. There was also a marked decrease in root length when compared with the control. The suppression of mitotic activity was often used in tracing cytotoxicity (Smaka-Kinel *et al.*, 1996). This is usually accompanied by an increase in cells with c-mitosis, sticky and abnormal chromosome orientation (Amin and Migahed, 2000). In this study a decrease in the mitotic index was found as the concentration of effluent increased. This indicates the cytotoxic effect of sewage effluent. The rate of aberration increased as the concentration increased. However the type of chromosomal aberration varied. Sticky chromosomes were most frequent in all concentrations. According to Odeigah *et al*. (1997b), sticky chromosomes are indicative of a highly toxic usually irreversible effect, leading to cell death. This could be responsible for the completely decayed roots found in 80% and 100% concentration. Chromosome stickiness is caused probably through immediate reactions with DNA during its inhibition periods, causing DNA-DNA or DNA-protein cross linking (Amin, 2002). Sticky chromosome might also be the result of incomplete replication of chromosomes by defective enzymes (Bennet, 1977). Sewage water also induced crochet hooks and c-tumors, which have been shown by other studies to be useful sign of toxicity (Amin, 2002). Crochet hooks might be due to presence of heavy metals while c-tumors effects are caused by several types of compounds, such as colchicines (Odeigah *et al.*, 1997a).

Results obtained in this study show that sewage effluent induced chromosomal aberration through interactions with DNA and proteins leading to chromosome stickiness, mitotic disturbances and cell damage. This result is similar to the findings of Odeigah *et al. (*1997b) in which roots of *Allium cepa* exposed to effluent from industrial wastes resulted to cells with chromosomal aberration.

The impacts of using sewage effluent as fertilizer on the environment are difficult to predict. However positive result in *Alliums* test should be considered a signal of warning as this may constitute risk to environment and human health. Therefore it is recommended that sewage water be treated before they are used as fertilizer so that safety of humans would be achieved.

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The Source of the Peano Axioms

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Abstract: The aim of this paper is to improve the conception of the natural numbers which is represented by the Peano axioms by introducing a non-arithmetical axiom. This way it can be demonstrated that if Ockham's razor is a correct principle then the terms 'zero', 'number' and 'immediate successor of' all spring from the same source. The multiplication of this source into three separate terms is upheld by the formal language but unnecessary for the clear mind.

Key words: Peano axioms; Ockham's razor; conception; non-arithmetical component; natural numbers

1. Introduction

Under consideration will be the five axioms related to the arithmetic of natural numbers (axiomatised by Giuseppe Peano in 1889) which are formulated with three undefined terms; 'zero', 'number' and 'immediate successor of', acquaintance with these terms being assumed [Nagel and Newman 1958 p.81]. These five axioms, hereafter called Peano axioms, can be stated as follows:

1. Zero is a number.
2. The immediate successor of a number is a number.
3. Zero is not the immediate successor of a number.
4. If the immediate successor of a number and the immediate successor of a number are the same then these numbers are also the same.
5. If a property belongs to zero, and also to the immediate successor of every number that has the property, then this property belongs to all numbers.

The following three statements will be examined:

I. Zero is not the immediate successor of a number.
II. Zero is the immediate successor of a number.
III. Zero is the immediate successor of not a number.

It is trivial that statement I is true because it follows immediately from axiom number 3. The falsehood of statement II is also clear because it is the negation of axiom number 3 and therefore contradicts this axiom. Statement III does not contradict the axioms as formulated here but this statement can neither be derived from these axioms, i.e., independent of these axioms. The meaningfulness of this statement, though, really depends on the assumed acquaintance with the undefined term 'immediate successor of'. Can statement III be accepted as sensible with respect to the conception of the natural numbers and if so, can statement III be accepted as a sentence with respect to the language of the formal system based upon the Peano axioms?

2. Discussion

The transformation of the conception of the arithmetic of natural numbers into a formal system can only be achieved by carving away all statements that do not obey the formality of this formal system. Apparently it is possible to find a meaningful statement (statement III) expressed in the vocabulary (calculus) of the formal system that does not obey the formation rules of this formal system, i.e., it cannot be formulated within arithmetic. This statement can be considered meaningful with respect to the conception of the natural numbers because either accepting statement III as true or as false results into a different representation of the conception of the natural numbers all be it in the non-arithmetical component. This means that either statement III or its negation is acceptable by the clear mind as sensible but neither statement III nor its negation is acceptable by the formal system as a sentence. In this case sense would appear for the formal system as non-sense.

In order to place the non-arithmetical component expressed by statement III or its negation onto the formal system one could add statement III or its negation to the list of axioms and this way extend the formal system. The system that is constructed this way can no longer be considered completely formal (unless the formation rules are adjusted) because it includes an informal sentence (statement III). This constructed system has the same vocabulary, the same rules of reasoning and an extended list of axioms. With respect to the formation rules it can be noted that this system has a formal language which reflects the conception of the arithmetical component of the natural numbers as well as an informal language which reflects the conception of the non-arithmetical component of the

natural numbers. Hence, the extension of the formal system with statement III or its negation results into a non-formal system.

There are two possible ways of extending the formal system with respect to statement III; statement III can be considered true or false. If statement III is taken to be false then the negation of statement III 'Zero is *not* the immediate successor of not a number' can be added to the Peano axioms. If statement III is taken to be true then statement III itself can be added to the Peano axioms. What happens in the latter case, however, is quite interesting because this new axiom reveals that zero is an immediate successor, all be it of not a number. So, in this extended system all numbers are immediate successors and all immediate successors are numbers. This means that the term 'number' may be substituted for the term 'immediate successor'. If this substitution is realised then statement III expresses that 'zero is the immediate successor of not an immediate successor'. Hence the term 'zero' can be substituted for the term 'the immediate successor of not an immediate successor'. The total result of these two substitutions makes it possible to formulate the axioms of this extended system (in total five axioms instead of six because statement III has been encoded within the substitutions) as follows:

i. The immediate successor of not an immediate successor is an immediate successor.
ii. The immediate successor of an immediate successor is an immediate successor.
iii. The immediate successor of not an immediate successor is not the immediate successor of an immediate successor.
iv. If the immediate successor of an immediate successor and the immediate successor of an immediate successor are the same then these immediate successors are also the same.[1]
v. If a property belongs to the immediate successor of not an immediate successor, and also to the immediate successor of every immediate successor that has the property, then this property belongs to all immediate successors.[2]

Both these two possible extensions (statement III is true, statement III is false) represent the same conception with respect to the arithmetic of natural numbers which means that these two theories make exactly the same predictions with respect to the arithmetical component of the natural numbers, i.e., the theorems of both extensions that are the same can be formulated within arithmetic. So both theories predict the same observable facts. It is only with respect to the non-arithmetical component of the natural numbers that these two extensions represent a different conception which means that the theorems of both extensions that are different cannot be formulated within arithmetic. It now merits to be reminded of Ockham's razor (named after William of Ockham also spelled Occam) which is the principle that entities should not be multiplied unnecessarily, or, if two competing theories make exactly the same (observable) predictions then the simpler one (less assumptions, postulates) is better. If one is allowed to invoke upon Ockham's razor then of these two competing theories that make exactly the same observable predictions the non-formal system which is based upon the axioms i-v is considered better than the non-formal system which is based upon the Peano axioms and the negation of statement III because the latter has just like the Peano axioms an *assumed* acquaintance with three undefined terms whereas the former has an *assumed* acquaintance with only one undefined term; 'immediate successor'.

3. Conclusion

Peano's formal conception of the arithmetic of natural numbers is carved out of a non- formal system which represents a more complete conception of the natural numbers, that is, both the arithmetical component and the non-arithmetical component. If Ockham's razor is a correct principle then the non-formal system that is based upon the axioms i-v is considered better and reveals that the terms 'zero', 'number' and 'immediate successor of' all spring from the same source but differ in name. The multiplication of this source into three separate terms is upheld by the language of the formal system. The clear mind, however, which is undisturbed by the formation rules can conceive[3] this unnecessarily multiplication.

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[1] This formulation simply reveals that distinct immediate successors have distinct immediate successors.
[2] It appears that this axiom has been derived from the following more complete formulation: 'If a property belongs to the immediate successor of not an immediate successor *that has not the property*, and also to the immediate successor of every immediate successor that has the property, then this property belongs to all immediate successors.'
[3] Statement III may also be conceived with the idea that zero arises from nothingness. Hence, zero immediately succeeds nothingness which is not a number.

The Impact of Maternal Micronutrient Levels on Risk of Offspring Neural Tube Defects in Egypt

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Abstract: Neural tube defects (NTD) are important causes of infant mortality. Poor nutrition was essential factor for central nervous system deformation. Mothers gave NTD offspring had abnormal serum levels of micronutrients. The present research was designed to study the effect of maternal micronutrient levels and oxidative stress on the incidence of NTD in offspring. The study included forty mothers; twenty of them of 30.9±7.28 years had conceived fetuses with NTD were considered as cases; and twenty mothers of 28.2 ± 7.82 years with healthy neonates. We determined se rum vitamin B_{12} and folic acid by using radioimmunoassays. Also, serum zinc was assessed using atomic absorption spectrophotometry. While serum copper and iron were measured colorimetrically and serum ceruloplasmin was analyzed by radialimmunodiffusion. Cases showed significantly lower levels of folic acid, vitamin B_{12} and zinc ($P \leq$ 0.0005, 0.01, 0.01 respectively) than that of the control. Concentrations of copper, ceruloplasmin, and iron were markedly increased in cases as compared to controls ($P \leq 0.01$, 0.01, and 0.05 respectively). In conclusion, the current study clearly indicated the etiology of NTD cannot be explained with one strict etiologic mechanism, on the contrary, an interaction among maternal nutritional factors and oxidative stress would explain these anomalies. Vitamin B_{12}, folic acid, and zinc supplementations should be considered for further decrease in the occurrence of NTD. Preventing excess iron during pregnancy favors better pregnancy outcomes. Report and Opinion. 2009;1(6):45-50]. (ISSN: 1553-9873).

Key words: Vitamin B_{12}, folic acid, zinc, copper, iron, ceruloplasmin, oxidative stress, neural tube defects.

1. Introduction:

Neural tube defects are important causes of infant mortality and childhood morbidity. The development and closure of the neural tube are normally completed within 28 days after conception, before many women are aware that they are pregnant. It's generally accepted that neutral tube defects are caused by the failure of the neural tube to close, although it has also been suggested that a closed tube may reopen (Campbell and Sohal, 1990). Spina bifida and an encephaly are the most common forms of neural tube defects (Birnbacher *et al*, 2002). Over years, epidemiologic studies have been instrumental in elucidating the causes of NTD in humans. Overall, these studies have suggested that environmental and genetic factors have a joint role in the causation of NTD. Nutrition may interact with a person's genetic make up. Furthermore, the effect of poor nutrition may be magnified in the developing embryo, where active cell proliferation occurs at a time when access of nutrients is limited (Botto *et al*, 1999). Therefore, poor nutrition was suggested to be an important risk factor for central nervous system malformations and women gave birth to babies with NTD had low serum levels of micronutrients (Jiang, 1991). Several groups have reported lower vitamin B concentrations and folate in particular in mothers of infant with NTD (Steegers-Theunissen, 1995). Therefore, folic acid supplementation is recommended for women who are planning to become pregnant as well as pregnant women (Lee et al, 2005 and Michels et al, 2008) because folate nutriture at periconception is critical for preventing NTD (Ryan-Harshman and Aldoori, 2008).Vitamin B_{12} is a part of coenzymes present in all body cells and is essential in the synthesis of DNA. It works closely with folic acid.

Minerals have also been implicated in the pathogenesis of NTD. Lower maternal serum and hair concentrations of zinc were associated with many anomalies (Srinivas *et al*, 2001). Iron deficiency is the most common nutrient deficiency in pregnant women and has been linked to negative impacts on the fetus,
women with NTD affected pregnancies had minimal or no ferritin (Felkner *et al*., 2005). Iron requirements during pregnancy are not easily fulfilled through diet alone, thus, it is recommended that pregnant women take iron supplements (Groenen *et al*., 2004). Copper deficiency during pregnancy is associated with multiple developmental defects that can affect the central nervous system and induce teratogenesis in the offspring (Keen *et al*., 2003 and Beckers

Trapp *et al*., 2006).

Evidence about the preventive effects of nutrients other than folate on the occurrence of NTD is scarce. Also, many studies have examined the impact of single nutrients on NTD risk and the role of nutrients in combination has received much less attention. Therefore, the aim of the current study was to investigate possible nutritive risk factors for the occurrence of NTD and some of their inter-correlations in women with normal and NTD infants at delivery.

2. Subjects and Methods:

(1) Subjects

The present study involved 40 mothers randomly chosen from the Neonatology unit of Maternity Hospital, Ain Shames University; twenty of them had neonates with neural tube defects. The mean age of them was 30.9 ± 7.28 year, their mean gestational age was 37.1 ± 2.92 weeks. The other twenty mothers had healthy neonates. Their mean age was 28.24 ± 7.82 year and their mean gestational age was 38.3 ± 1.12 weeks. Mothers never administered any supplement, during gestation. All studied mothers were subjected to full history with laying stress on their obstetric history including grandparity, gestational age, consanguinity and similar condition in the family. Also, attention was focused on their possible exposures to physical agents such as trauma, hyperthermia, radiation, infections and metabolic disarrangement such as diabetes mellitus or drugs. In addition, a dietary questionnaire was taken.

(2) Blood sampling and analytical methods

Venous blood samples were withdrawn immediately after delivery from fasting mothers; serum was separated by centrifugation and stored at -20°C for analysis. Simultaneous quantitative determinations of vitamin B_{12} and folate were done by radioimmunoassays (RIAs). ICN pharmaceticals simul TRAC, SNB radioassay kit, was used (Kubasik *et al*., 1979). Vitamin B_{12} and folate tracers, binders and standards were supplied in combined form. The pteriyoglutamic acid form of folate is used as both standard and tracer in an incubation mixture at pH 9.5. The two tracers [^{57}Cr] for vitamin B_{12}, and [^{125}I] for folate produce energies at levels which were easily separated by two-channel gamma counter. Serum zinc measurement was done by flame atomic absorption spectrophotmetry (Smith *et al*., 1979). Serum copper and iron levels were determined colorimetrically (Abe *et al*., 1989 and Williams *et al*., 1977; respectively) using commercially available kits. Sentinel CH. Italy and Elitech diagnostic, respectively. Moreover, ceruloplasmin concentrations were estimated by using radial immunodiffusion (RID) plates obtained from Biocientifica S.A. Argenting according to Verbruggen (1975).

(3) Statistical methods

All statistical analysis is performed with the use of SPSS computer program. Mean ± SD differences between cases (mothers had NTD neonates) and control (mothers had healthy neonates) were analyzed with a student's two tailed t. test. A probability value $P < 0.05$ was considered to be statistically significant, while that corresponding to $P < 0.01$ was considered to be highly significant.

3. Results

Table (1) indicates mean values of serum folic acid and vitamin B_{12} in mothers of healthy and NTD offspring. It shows that serum vitamin B_{12} concentrations in cases were significantly decreased than those in control ($P < 0.01$) and the percentage of decrease was 35.9. However, folic acid levels were significantly reduced in cases by 31.4%. Serum mean values of zinc, copper, ceruloplasmin and iron are represented in table (2). Mothers with NTD infants exhibited an extreme decrease in zinc level ($P < 0.01$) by 36.3% indicating a significant association between zinc level and presence of NTD. By contrast, the concentrations of copper, ceruloplasmin and iron were markedly increased ($P < 0.01$, 0.01 and 0.05 respectively). The percentages of increase were 31.8, 31.9 and 18.2 respectively. It is obvious from the percentages that those of copper and ceruloplasmin were nearly the same. Results indicated that there was a strong association between copper, ceruloplasmin and iron levels and incidence of NTD.

Table 1. Serum Folic Acid and Vitamin B_{12} Concentrations (Means ± S.D.) in Mothers of Healthy and NTD Offspring

Groups	Folic acid (ng/ml)	Vitamin B_{12} (pg/ml)
Mothers of healthy offspring (n=20)	10.5 ± 0.668	1070 ± 387
Mothers of NTD offspring (n = 20)	7.2 ± 0.346	685.5 ± 103**

** High significant difference at $P < 0.01$.
***Very high significant difference at $P<0.0005$.

Table 2. Comparison of Serum Trace Elements and Ceruloplasmin Levels (Means±S.D.) Between Mothers of Healthy and NTD Offspring

Groups	Zinc µg/dL	Copper µg/dL	Ceruloplasmin mg/dL	Iron µg/dL
Mothers of healthy offspring (n=20)	74.1 ± 4.1	95.6 ± 31.6	42.6 ± 13.8	84.4 ± 16.7
Mothers of NTD in offspring (n = 20)	47.2± 9.5**	126**±26.5	56.2**±17.4	99.8*± 27.8

* Significant difference at $P < 0.05$.
** High significant difference at $P < 0.01$.

4. Discussion

Neural tube defects are the most common congenital anomalies of the central nervous system resulting from failure of the neural tube to close in the first trimester. They result from multiple intrinsic and extrinsic factors (Botto *et al*., 1999). NTD are considered as important causes of infant mortality and childhood morbidity. Women of childbearing age are currently advised to consume 0.4 mg of folic acid per day to prevent NTD (Williams, 1995). In addition to folate, other nutrients such as vitamin B_{12}, zinc, and methionine may also influence NTD risk. Accordingly, this study was designed to evaluate micronutrient status in women with normal and NTD offspring at delivery.

Fetal growth and development are characterized by widespread cell division. Adequate folate is critical because of its role in DNA and RNA synthesis. Our data revealed that serum folic acid levels showed statistically significant reduction in mothers had NTD neonates as compared to those with healthy ones. This finding has been pointed out in different studies (Steegers - Theunissen, 1995 and Boyles *et al.,* 2008). But Weekes *et al*., (1992) and Villarreal *et al*., (2001) have pointed out that folic acid exhibited no change in NTD mothers. This discrepancy may be resulted from sampling variations. However, abnormal folate metabolism affects gene expression resulting NTD (Dunlevyl *et al.,* 2007 and Boyles *et al.,* 2008). Moreover, folate deficiency can lead to hyperhomocysteinemia (Van-wersh *et al*., 2002), which is a known risk factor for NTD (Vollset *et al*., 2000). Besides, an approximate doubling of the risk of spina bifida has been associated with mutations in the gene for methylenetetrahydrofolate reductase which can be prevented by folic acid supplementation (Michels *et al.,* 2008).

Maternal vitamin B_{12} levels were significantly lower in cases as compared to control mothers. Our results are in accordance with those of Kirke *et al*., (1993) and Wald *et al*., (1996). Many clinical studies have been generated cobalamin metabolism in an effort to identify the biochemical and the genetic bases of neural tube defects. Cobalamin is a part of coenzymes present in all body cells and is essential in the synthesis of DNA. Maternal cobalamin shortage resulting in a mild hyperhomocysteinemia that was also associated with spina bifida (Steegers-Theunissen *et al*., 1991 and Mills *et al*., 1996). Where the conversion of homocysteine to methionine is a critical step for neural tube closure. This methionine synthase pathway is dependent on folate coenzyme and vitamin B_{12} dependent enzyme (Gerhard and Duell, 1999). As for genes, initial findings indicate that the genetic contribution to NTD is likely to be complex. An approximate doubling of the risk of spina bifida has been associated with homozygosity for a common mutation in the gene for methylenetetrahydrofolate reductase (MTHFR), the C677T allelic variant (Rozen, 1997) which encodes an enzyme with reduced activity. Reduced maternal blood levels of vitamin B_{12} increased this mutation (Christensen *et al*., 1999). However, the reduced levels of vitamin B_{12} may be due to low dietary intake, as it is present only in animal sources, and/or its malabsorption in cases. Consequently, increasing maternal vitamin B_{12} is of benefits to reduce NTD (Kirke *et al*, 1993).

Pronounced zinc depletion has been reported in women with NTD fetuses (Jiang, 1991 and Cengiz *et al*., 2004). In the present study, we observed that zinc changes are consistent with those of the previous authors. Zinc has the critical role in DNA synthesis and regulation of gene expression by binding to DNA and influencing the transcription of specific genes. Also, it is a major regulatory ion in the metabolism of cells especially in tissues with a high cellular turnover (Black, 1998). Moreover, zinc affects folate status as a cofactor for methionine synthase and □-glutamyl hydrolase (Groenen *et al*, 2004). Therefore, it is conceivable that zinc decreased levels may contribute in the risk of NTD.

It is revealed from our data that cases had markedly higher serum copper concentrations as compared to control mothers. Our results are

consistent with previous reports (Cengiz *et al*., 2004). Besides, they demonstrated a negative correlation existed between serum zinc and copper levels in mothers with NTD enfants. There is increasing evidence that significant interactions occur between and among essential nutrients at levels that are not considered to be toxic. High dietary zinc caused a pronounced copper depletion in rats even when adequate copper was included in the diets (Sundaresan *et al*., 1996). Consequently, the decreased levels of zinc in our cases were responsible for enhancing intestinal copper absorption with concomitant increase in its serum levels. About 90% of plasma copper is found in ceruloplasmin in normal mammals and highly significant correlations have been demonstrated between ceruloplasmin level and copper level. Our finding that high serum ceruloplasmin level was accompanied to increased serum copper levels in cases is in agreement with the previous authors (Davis and Mertz, 1987). Moreover, serum ceruloplasmin concentration was strongly influenced by an interaction between zinc and copper. As dietary zinc increased with adequate copper levels, serum ceruloplasmin concentrations decreased (Sundaresan *et al*, 1996).

There is abundent evidence showing that maternal oxidative stress may interfere with neural tube closure (Marzullo & Fraser, 2005, Zhaow-Mosley *et al*., 2006 and Benko & Brodland, 2007), via inhibiting the expression of Pax-3 gene that is essential for such closure (Chang *et al*., 2003 and Loeken, 2006). Therefore, the increase in copper and ceruloplasmin levels in our study may reflect a compensatory defense mechanism of the body against oxidative stress (Johnson *et al*., 1992).

Our data revealed that the iron level was significantly higher in cases than in control. Although iron is an essential nutrient for growth and development, high tissue iron concentrations have been associated with the development and progression of several pathological conditions (Fraga & Oteiza, 2002). Excess iron is a highly effective promoter of lipid peroxidation and free radical generation which result in molecular, cellular, and tissue damages (Kontoghiorghes *et al*., 2000). However, the increase in iron level may be explained by the competitive interactions between iron and zinc (Fairweather-Tait & Thouthon, 1989). It's noticed that the increase in iron concentration was followed by a rise in ceruloplasmin concentration, as ceruloplasmin is an important extracellular antioxidant that function coordinately with transferrin by its ferroxidase activity to promote iron binding and prevention of metal-catalyzed free radical reactions (Johnson *et al*., 1992).

From the foregoing results, it could be concluded that deficiencies of vitamin B_{12}, folic acid and zinc,
in addition to loss of oxidant/antioxidant balance may participate in the higher incidence of NTD pregnancies. The risk of excess supplementation by iron and its potential adverse effects should be considered. Factors other than dietary ones would explain these anomalies.

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Zooplankton Dynamics and Chlorophyll *a* Concentrations at The Tomaro Creek In Relation To Water Quality Indices

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ABSTRACT

Aspects of zooplankton dynamics at the Tomaro creek in relation to water quality characteristics were investigated for a period of six months (October, 2007 to March, 2008). Rainfall distribution and possibly tidal seawater inflow were the key factors that govern the variation in zooplankton distribution, species diversity, chlorophyll *a* concentration and water quality indices of the creek. Salinity and rainfall recorded a strongly negative correlation (r = -0.85). Chlorophyll *a* concentrations were higher in the dry season months than in the wet season. Values range between 6 and 22µg/L for the study. A total of 18 species from 15 genera were recorded for the zooplankton and the population was grouped under 2 phyla and 3 classes. The classes observed were Crustacea, Mysidacea and Scyphozoa. The class crustacea with the subclass Copepoda was the most dominant, accounting for 59.15% of the total species recorded. The juvenile stages accounted for 39.02% of the total species recorded. The Shannon-Wiener index (Hs) was highest (0.85) in February, 2008 and lowest (0.22) in November, 2007. The Species richness index (d) was generally high (>0.26), while species evenness was low (<0.97) throughout the period of study. [Report and Opinion 2009;1(6):51-64]. (ISSN: 1553-9873).

INTRODUCTION

An intricate network of creeks, rivers and lagoons exist in South-western Nigeria which eventually connects to the sea via the Lagos harbour. Flood waters associated with rainfall are known to enrich the coastal environment, dilute its ionic concentration and break down existing environmental gradients (Nwankwo, 1996; Onyema, 2008). The coastal waters of South-western Nigeria include a system of lagoons which receives a number of large rivers and creeks draining more than 64,000km^2 of the country (Chukwu, 2002).

Owning to the seasonal distribution of rainfall, the lagoon system and creeks experiences seasonal flooding which dilutes the ionic concentration of the coastal waters and introduces a lot of detritus, nutrients, as well as land based potential environmental pollutant from land based activities which includes domestic and industrial effluents, urban storm run-off agricultural land run-off and shipping activities (Akpata *et al*., 1993; Nwankwo, 1993; Onyema *et al*., 2003, 2007; Onyema and Nwankwo, 2006).

According to Nwankwo (2004), an important ecological ramification of increasing population pressure, poor sewerage system, industrialization and poor waste management in Nigeria coastal area is that pollutants freely find their way unabated into our coastal waters through drains, canals, rivers, creeks and lagoons that act as conduits. The increase in available nutrients coupled with reduced current speed causes an increase in phytoplankton population and hence an increase in zooplankton.

Studies on the planktonic components of Lagos coastal waters have concentrated more on the phytoplankton components (Hendey, 1958; Nwankwo, 1984, 1986, 1988). Existing information on creeks, chlorophyll *a* levels and zooplankton spectrum of South Western Nigeria are scanty. The aim of the project was to investigate the aspects of zooplankton dynamics and chlorophyll *a* at the Tomaro creek in relation to water quality situations.

MATERIALS AND METHODS

Description of Study Site

The Tomaro creek is linked to the Badagry creek which joins the Lagos lagoon at the harbour in South-western Nigeria. The creek is located in Amuwo Odofin L.G.A of Lagos State and is bordered by a predominantly rural and sparsely populated community showing very little signs of urbanisation. The study site located at the Tomaro creek lies around Longitude 03^0 22' 52E and Latitude 06^0 25' 43N. Creeks and lagoons are common hydrological features of South-western Nigeria and form part of the numerous ecological niches associated with the Nigerian coastal environment (Chukwu and Nwankwo, 2004; Onyema, 2007).

This site experiences tidal influences from the adjacent Badagry creek and lagoon to which it opens. As with the lagoon, rising tide ushers in high water levels, which increase salinity. At low tide, the water level and salinity fall exposing tidal flats in the furthest extremes.

The Tomaro creek area falls within the rainforest zone which experiences a well marked dry (December -

April) and wet (May - November) seasons. Tidal oscillation along the creeks length is of the semi-diurnal tidal regime and the same as the whole Gulf of Guinea (West African coast). The effect of the tide is more discernable in the dry season and decreases inland resulting in characteristics environmental and biota gradients (Nwankwo and Akinsoji, 1989). The entire length of the creek is covered by a luxuriant growth of mangroves species with Red Mangrove (*Rhizophora racemosa*) being the dominant member. Tidal mudflats are observable at low tide and especially in the dry season. There is also the presence of an abundance of floating water hyacinth (*Eichhornia crassipies*) at the edges of the creek in the wet season.

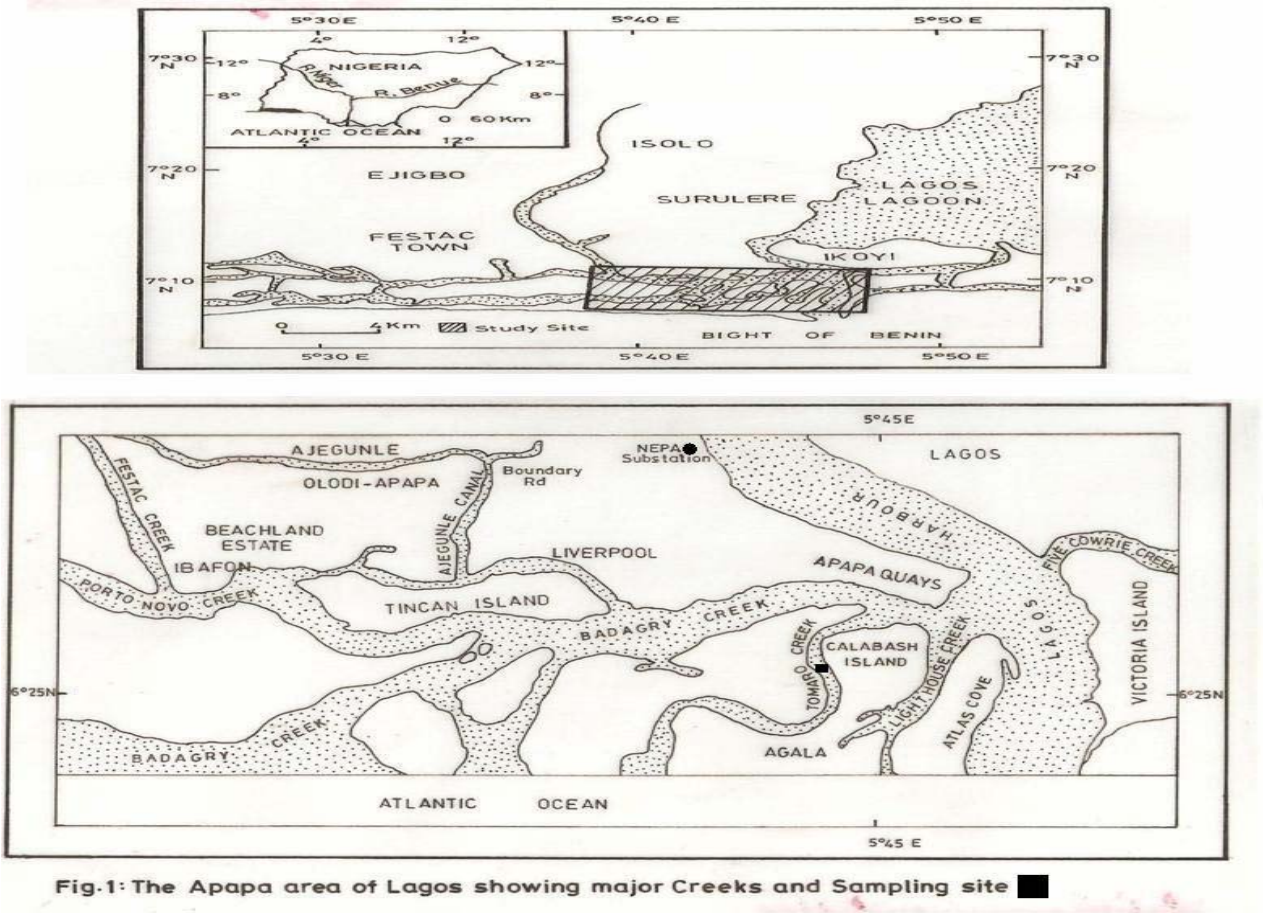

Fig.1: The Apapa area of Lagos showing major Creeks and Sampling site ■

Collection of Water Samples

Monthly surface water samples for physico-chemical analysis were collected for six months (October, 2007 – March, 2008**)** with 500ml plastic containers with screw cap at the Tomaro creek using an outboard engine powered boat.

Collection of Plankton Samples

A horizontal plankton haul was made on each trip with a standard plankton net of 55µm mesh size towed at low speed (4 knots) for five minutes. The plankton samples were concentrated and stored in 500ml plastic containers (properly labelled showing the time, date of collection and study site). Samples were preserved in 4% unbuffered formalin which was then transferred to the laboratory for further analysis.

Analysis of water quality parameters.

Table 2: Summary of method/device for the estimation of water quality parameters.

	Parameter/ Unit	Method / Device	Reference(s)
1	Air temperature (^oC)	Mercury – in – glass thermometer	Nwankwo (1984)
2	Water temperature (^oC)	Mercury – in – glass thermometer	Onyema (2008)
3	Transparency (cm)	Secchi disc method	Onyema (2008)
4	Depth (cm)	Graduated pole	Brown (1998)
5	Rainfall (mm)	Acquired from NIMET, Oshodi, Lagos	
6	Total Dissolved Solids (mg/L)	Cole Palmer TDS meter	
7	Total Suspended Solids (mg/L)	Gravimetric method	APHA (1998)
8	Chloride (mg/L)	Argentometric method	APHA (1998)
9	Total hardness (mg/L)	Titrimetric method	APHA (1998)
10	pH	Electrometric / Cole Parmer Testr3	
11	Conductivity (µS/cm)	Philip PW9505 Conductivity meter	
12	Salinity (‰)	HANNA Instrument	APHA (1998)
13	Alkalinity (mg/L)	Titration method	APHA (1998)
14	Acidity (mg/L)	Titration method	APHA (1998)
15	Dissolved oxygen (mg/L)	Titration method	APHA (1998)
16	Biological oxygen demand (mg/L)	Incubation and Titration	APHA (1998)
17	Chemical oxygen demand (mg/L)	Titration method	APHA (1998)
18	Nitrate – nitrogen (mgL)	Colorimetric method	APHA (1998)
19	Phosphate – phosphorus (mg/L)	Colorimetric method	APHA (1998)
20	Sulphate (mg/L)	Turbidimetric method	APHA (1998)
21	Silica (mg/L)	Colorimeter (DR2010)	APHA (1998)
22	Calcium (mg/L)	Titrimetric method	APHA (1998)
23	Magnesium (mg/L)	Titrimetric method	APHA (1998)
24	Copper (mg/L)	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application methods (2002)
25	Iron (mg/L)	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application methods (2002)
26	Zinc (mg/L)	Atomic Absorption Spectrophotometer Perkin Elmer 5000 AAS	Perkin Elmer Application methods (2002)
27	Chlorophyll a (µg/L)	Florometric method	APHA (1998)

Biomass In Terms of Numbers Using Counting Methods

Plankton sample were allowed to settle in the lab for 2hrs and concentrated to 20ml. For each settled sample, 5 drops of well mixed sample were investigated. On each occasion, one drop of sample was thoroughly investigated using the Drop Count Method described by Lackey (1938). For each drop five transect were investigated by moving the stage at different position under a Carl Zeiss monocular microscope.

Zooplankton species were observed, identified and drawn using text. Several relevant keys and illustrations: Newell and Newell (1966), Wimpenny (1966), Olaniyan (1975), Gibbons (2001) and Waife and Frid (2001) were consulted to confirm identification.

Community Structure Analysis

Species diversity index (Shannon-Wiener, 1963), Species richness (Margalef, 1951); Evenness or equitability indices (Pielou, 1975) and Simpson Dominance index were used to estimate the zooplankton biodiversity.

Species Richness Index (d)

This is also known as the species diversity index. The species richness (Margalef, 1951) was given by the equation.

$$d = \frac{S - 1}{\ln N}$$

Where d = Margalef richness index or Species diversity index, S = Number of species in the population, N = Total number of individuals in species.

Shannon -Wiener Index (Hs)

This was proposed by Shannon-Wiener (1963) and it is given by the equation:

$$Hs = \frac{N \log N - (\sum Pi \log Pi)}{N}$$

Where Hs = Shannon-Weiner diversity index, $\sum$ = Summation, i = count denoting i^{th} species ranging from 1 to n. Pi = proportion that the i^{th} species represent to the total number of individuals in the Sampling space.

Species Evenness (j)

Species equitability or evenness was determined by the equation:

$$j = \frac{Hs}{\log_2 S}$$

Where j = equitability index, Hs = Shannon-Weiner diversity index, S = number of species in the population.

Simpson's Dominance Index (C)

Simpson's dominance index by Simpson (1949) using the equaton:

$$C = \sum \left(\frac{n_i}{N}\right)^2$$

ni = the no. of individuals in the iths species, Ni = the total no. of individuals.

Correlation Coefficient (r)

The correlation between zooplankton abundance and some environmental variable (Temperature, salinity, total weekly rainfall and nutrient levels (Phosphorus and Nitrates] was determined by Spearman Rank correlation analysis and it is given by the equation:

$$r = \frac{1 - 6 \sum D^2}{n(n^2 - 1)}$$

Where r = correlation coefficient, $\sum D^2$ = sum of squares of difference of the ranks, n = number of weeks.

RESULTS

The variation of the water quality indices of the Tomaro creek between October 2007 and March 2008 are represented in Table 1. The air temperature variation recorded showed minimal variation and ranged between 27 and 30.5^{0}C. The highest temperature value (30^{0}C) was recorded both in February and March respectively while the lowest value (27^{0}C) was recorded in January.

Table 1: Monthly Variations in the Water Quality Indices at Tomaro Creek (October 2007- March 2008).

PARAMETERS		MONTHS							
		OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	MEAN	STD. DEV.
1	Air Temperature (°C)	30.5	30	31	27.8	26	26	28.13	±2.08
2	Water Temperature(°C)	29.5	28.2	29.1	27	30.5	30.5	27.98	±1.64
3	Transparency(cm)	25	61.8	98.5	128.5	174	174	111.17	±44.18
4	pH at 26°C	7.32	7.38	7.33	7.6	7.67	7.53	7.47	±0.15
5	Conductivity (µS/cm)	3270	5730	19930	31000	38700	37300	22655	±15564
6	Rainfall (mm)	87.9	19.5	8.9	74.4	17.6	44.5	24.5	±13.89
7	Total Suspended Solids (mg/L)	32	16	16	102	33	240	73.17	±87.76
8	Total Dissolved Solids (mg/L)	1716	2530	8220	16880	20472	19340	11526.3	±8459.89
9	Salinity(‰)	1.71	3.1	11	17.4	23.2	21.1	12.92	±9.15
10	Acidity (mg/L)	1.8	2.9	6.6	6.5	7.5	7.5	5.47	±2.48
11	Alkalinity (mg/L)	70	70	385	281.2	1190	1255.1	541.88	±541.59
12	Total Hardness (mg/L)	764.5	843	2780	4173	7645	7880	4014.25	±3171.5
13	Calcium (mg/L)	27.8	55.6	170	511.5	2080.1	2001.1	807.68	±970.72
14	Magnesium (mg/L)	170	166.3	574.4	723.9	583.2	590.4	468.03	±238.69
15	Zinc (mg/L)	0.006	0.008	0.02	0.007	0.011	0.022	0.01233	±0.01
16	Iron (mg/L)	0.11	0.14	0.16	0.19	0.1	0.25	0.15833	±0.06
17	Copper (mg/L)	0.002	0.003	0.002	0.003	0.005	0.004	0.0032	±0.00
18	Chloride (mg/L)	725	1450.3	5220	8120	12125	11000	6440.05	±4795.2
19	Nitrate (mg/L)	5	3	3.2	4.8	0.7	4.1	3.466	±1.58
20	Sulphate (mg/L)	25.5	116.5	480	610.2	1112.5	1150	582.45	±477.83

21	Phosphate (mg/L)	1.8	0.6	0.25	3.5	1.1	0.3	1.2583	±1.24
22	Silica (mg/L)	1.6	3.6	2.9	4.2	3.4	3	3.12	±0.87
23	Biochemical Oxygen Demand (mg/L)	80	24	50	24	25	33	39.33	±22.29
24	Chemical Oxygen Demand (mg/L)	260	128	505	118	188	68	211.17	±158.33
25	Dissolved Oxygen (mg/L)	4.2	3.6	4	3.8	5.4	4.8	4.3	±0.68
26	Chlorophyll-*a* (µg/L)	9	15	12	22	16	6	13.33	±5.65
27	Species diversity (S)	2	2	4	7	10	8	5.5	±3.33
28	Species abundance (N)	50	50	125	140	170	275	135	±84.14

The surface water temperature ranged between 25.9 and 30.5^{0}C. The highest temperature value (30.5^{0}C) was recorded in March while the lowest temperature value (25.9^{0}C) was recorded in February. The lowest value for air temperature recorded during the wet season was 30.5°C recorded in October and lowest value during the dry season was 26°C recorded in February and March. The average value is 27.98 and standard deviation of ±1.64. The transparency level showed a very wide range of variations between 25 and 174cm. The transparency level showed that the highest transparency level value of 174cm was recorded in February. The lowest value during the wet season was 25cm recorded in October and 128.5cm during the dry season in January, while the lowest transparency level value of 25cm was recorded in October with an average value is 111.17 and standard deviation of ±44.18. The total suspended solids range between 16 and 240mg/L. The lowest value recorded was both 16mg/L in November and December respectively and highest value recorded 240mg/L was in March. The total dissolved solids steadily increased greatly with the months and the values ranged between 1716 and of 20472mg/L. The highest value of 20472mg/L was recorded in February, while the lowest value of 1716mg/L was recorded in October.

The rainfall data showed a distinct monthly variation ranging between 8.9 and 87.9mm. The highest amount of rainfall 87.9mm was recorded in October while the lowest amount (8.9mm) was recorded in December. The surface water pH showed minimal variations. The highest pH value (7.67) was recorded in February, while the lowest value (7.32) was recorded in October. The surface water acidity also showed monthly variations. The highest acidity value of 7.5mg/L was recorded in March while the lowest acidity value was 1.8mg/L recorded in October. The surface water alkalinity varied throughout the sampling period. The lowest value 70mg/L was recorded in October, while the highest value 1255.1mg/L was recorded in March. The lowest value during the wet season was 70mg/L recorded in October and 281.2mg/L during the dry season in January. Salinity values recorded showed that salinity of the water was low and the values ranged between 1.71 and 23.2‰. The month of October had the lowest salinity value of 1.71 while February had the highest salinity level of 23.2 ‰. The lowest value during the wet season was 1.71‰ recorded in October and 17.4‰ during the dry season in January. The conductivity values showed a very wide range in variations which increased constantly with the months. The highest value of 38700µS/cm was recorded in February while the lowest value of 3270µS/cm was recorded in October.

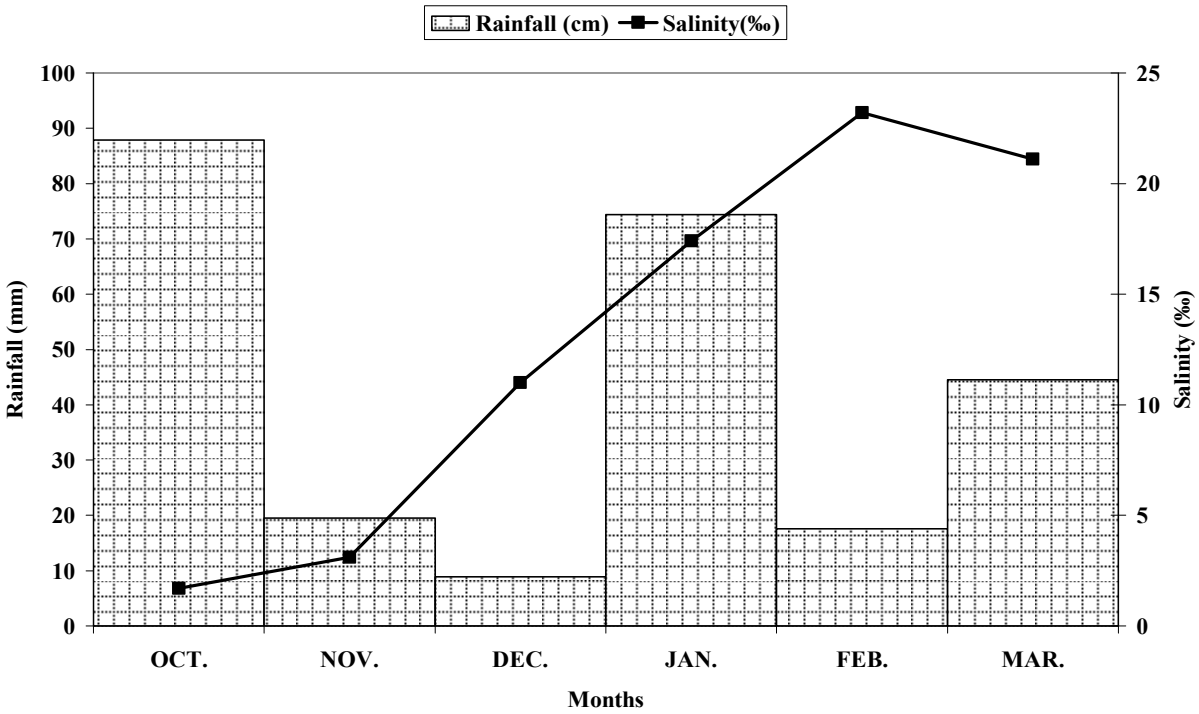

Fig. 5: Monthly variations in Rainfall and Salinity at the Tomaro creek (October, 2007 to March, 2008).

The Dissolved oxygen (DO) showed minimal variations ranging from 3.6 to 5.4mg/L. Lower levels of dissolved oxygen were recorded from November to January. The highest value (5.4mg/L) was recorded in February and the lowest value (3.6mg/L) which was estimated in November. The Biochemical Oxygen Demand was highest at the beginning of the sampling period and decrease subsequently to the end of the sampling period with exceptions in the months of December which had a value of 50mg/L. The lowest value of 24mg/L was recorded in November while the highest value 80mg/L was recorded in October. The highest value of COD, 505mg/L was recorded in December while the lowest value (68mg/L) was recorded in March. The lowest value during the wet season was 128mg/L recorded in November and 68mg/L during the dry season in March. The total hardness values showed a steady increase with each sampling month and ranged between 764.5 and 7880mg/L. The highest value 7880mg/L was recorded in March, while the lowest value 764.5mg/L was recorded in October.

Surface water chloride values showed a steady increase in each month and a wide range of variations. The lowest chloride value of 725mg/L was recorded in October, while the highest value 1450.3mg/L was recorded in November. The calcium level ranged from 27.8 to 2080.1mg/L . The highest value 2080.1mg/L was recorded in February while the lowest value 27.8mg/L occurred in October. The calcium level ranged from 166.3 to 723.9 mg/L. The highest value723.9mg/L was recorded in January while the lowest value 166.3mg/L occurred in November. The nitrate–nitrogen concentrations ranged between 0.7and 5mg/L. The sulphide concentrations varied through 25.5mg/L recorded in October to 1150mg/L recorded in March. The phosphate–phosphorus concentrations showed monthly variations and these concentrations recorded showed that the highest value 3.5mg/L occurred in January, while the lowest value 0.3mg/L occurred in March.

The surface water silica concentrations showed distinct monthly variations. The highest silica concentration (4.2mg/L) was recorded in January, while the lowest concentration (1.6mg/L) was recorded in October. Copper values were between 0.002 and 0.005mg/L with an average value of 0.0032 mg/L . Iron showed fluctuation in concentration between 0.1mg/L to 0.25mg/L with an average value of 0.16 mg/L. Zinc values showed very minimal variations ranging from 0.006 to 0.022mg/L with an average value of 0.012 mg/L. The lowest value during the wet season was 0.02mg/L recorded in December and 0.007mg/L during the dry.

Chlorophyll *a* (µg/L)

Chlorophyll *a* concentration ranged from 6µg/L recorded in March to 22µg/Lrecorded in January. Average chlorophyll *a* concentration for the period of study was 13.33µg/l while standard deviation was ±

5.65. Chlorophyll *a* values were higher in the dry than the wet season.

Zooplankton diversity and abundance

The aspect of zooplankton dynamics species at the Tomaro creek between October, 2007 and March, 2008 is presented in Table 2.

A total of 13 zooplankton genera constituting 18 species were recorded throughout the study period. More taxa were observed in February and March compared to October and November. Two major phyla of zooplankton were identified at the Tomaro creek throughout the sampling period which are the Arthropoda and Cnidaria. The major orders represented were the Order - Copepoda and Order - Siphonophora. Of these, the phylum Arthropoda was the most abundant, accounting for 99% of the total species composition with the phylum Cnidaria recording 1% of the total species composition. Among the Arthropods, *Acartia discaudata* Giesbrecht (Calanoida) had the highest number of species (195 individuals were recorded). *Centropages furcatus* Dana, *Metridia longa* Lubbock (copepoda) ranked lowest with only 10 individuals each. Among the Cnidaria, there were some unidentified jelly fish in the Class - Scyphozoa.

With regard to the juvenile stage, nauplii, zoea, megalop, bivalve, gastropod larvae and fish eggs constituted the juvenile stages collected at the sampling site. The Nauplii larva had the highest number of individuals, accounting for 68% of the total juvenile stages composition while the bivalve and gastropod larva had the lowest number of individuals, accounting for 5% of the total composition respectively.

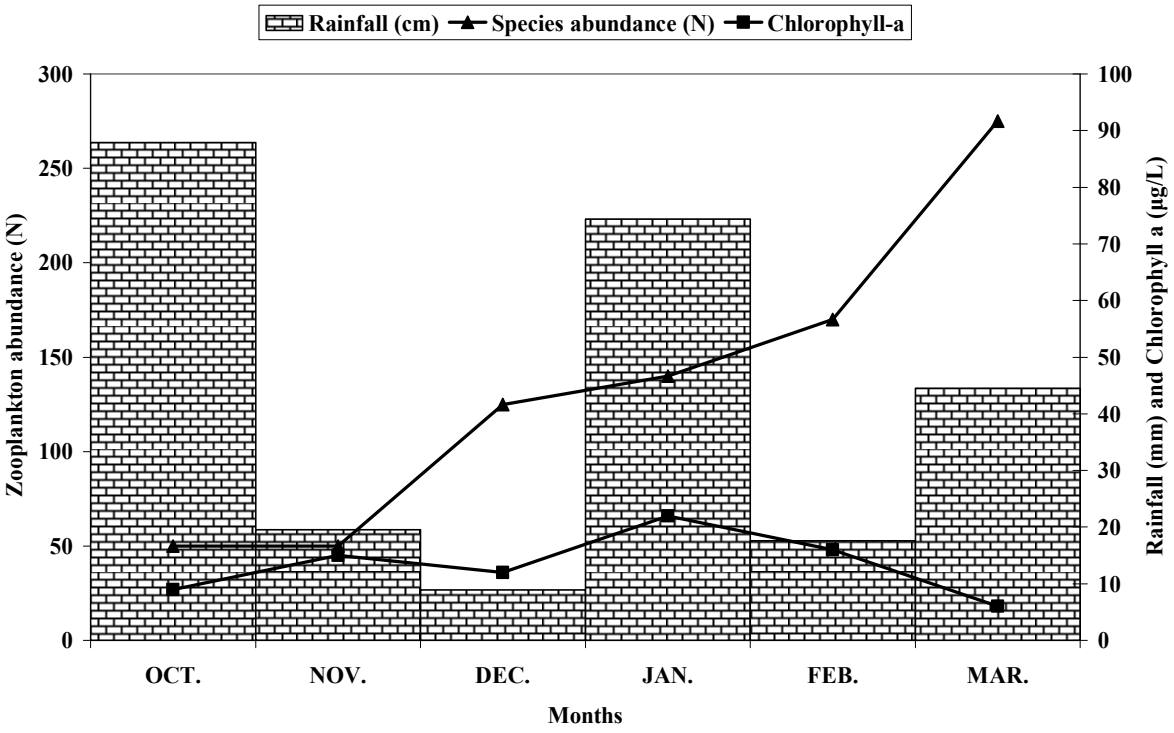

Fig. 10: Monthly variations in Rainfall, Chlorophyll *a* and Zooplankton abundance at the Tomaro creek (October, 2007 to March, 2008).

TABLE 2: SPECIES COMPOSITION AND ABUNDANCE (Cells/ml) OF ZOOPLANKTON AT THE TOMARO CREEK COLLECTED BETWEEN (OCTOBER, 2007 AND MARCH, 2008).

TAXA	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.
CLASS: CRUSTACEA						
SUB-CLASS: COPEPODA						
ORDER I: CALANOIDA						
FAMILY: ACARTIIDAE						
Acartia clausii Giesbrecht	20	-	-	-	10	-
Acartia discaudata Giesbrecht	-	10	70	50	25	35

Acartia tonsa Dana	-	40	-	-	20	75
FAMILY: METRIIDAE						
Metridia longa (Lubbock)	-	-	-	10	-	-
FAMILY: PARACALANOIDAE						
Calanus finmarchicus (Gunn.)	-	-	-	-	5	-
Centropages furcatus Dana	-	-	-	-	5	5
Paracalanus parvus Claus	-	-	10	-	60	25
ORDER II: CYCLOPIDA						
Cyclopina longicornis Claus	-	-	-	-	10	-
CLASS: MYSIDACEA						
FAMILY: MYSIDAE						
Mysis sp	-	-	-	-	5	-
JUVENILE STAGES						
Lucifer zoea larva	-	-	10	-	-	-
Megalopa larva	-	-	-	15	10	10
Nauplii larva of Barnacle	-	-	35	40	-	95
Nauplii larva of copepods	-	-	-	-	20	20
Zoea larva	-	-	-	10	-	-
PHYLUM: CNIDARIA						
CLASS: SCYPHOZOA						
ORDER: SIPHONOPHORA						
Unidentified jelly-fish	-	-	-	10	-	-
JUVENILE STAGES						
Bivalve larva	-	-	-	5	-	-
Fish eggs	30	-	-	-	-	-
Gastropod larva	-	-	-	-	-	10
Total species diversity (S)	**2**	**2**	**4**	**7**	**10**	**8**
Total zooplankton abundance (N)	**50**	**50**	**125**	**140**	**170**	**275**

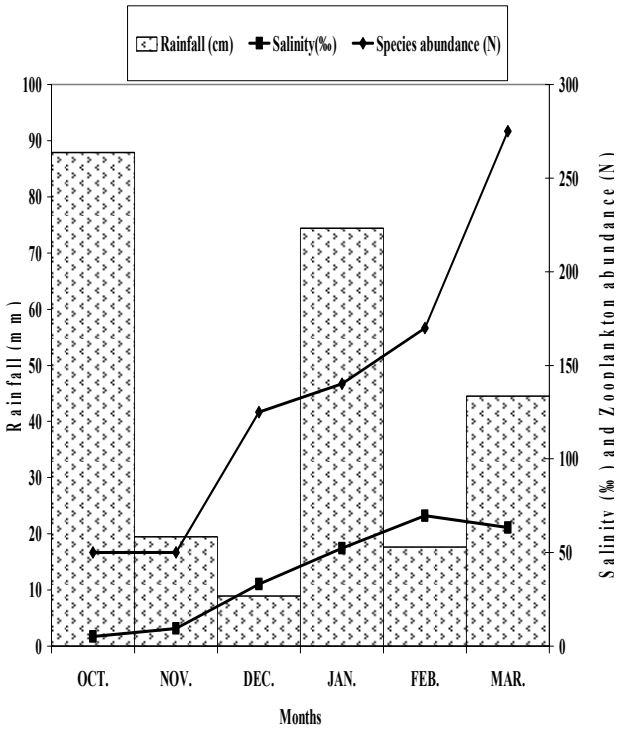

Fig. 11: Monthly variations in Rainfall, Salinity and Zooplankton abundance at the Tomaro creek (October, 2007 to March, 2008).

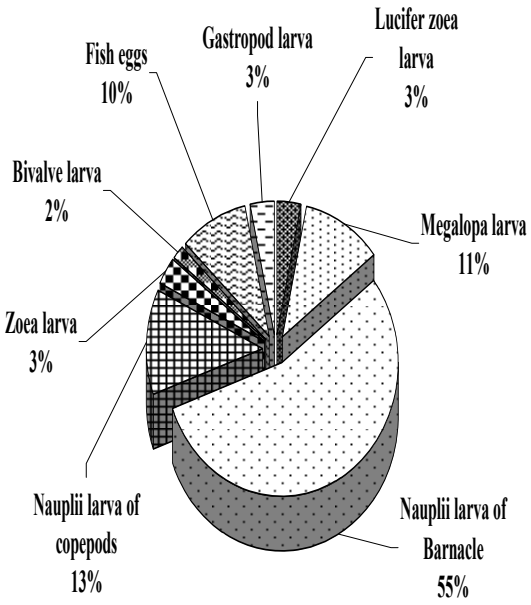

Fig. 13: Relative abundance of juvenile stages at the Tomaro creek (October, 2007 to March, 2008).

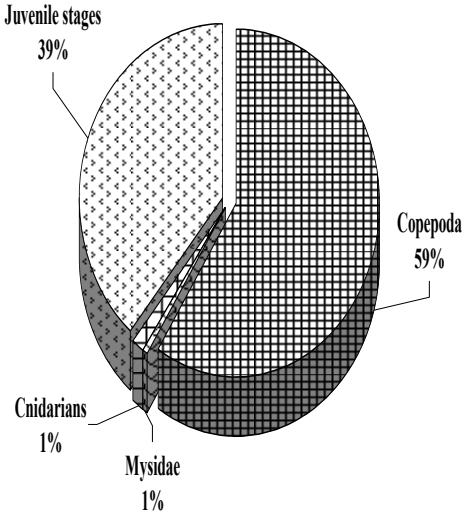

Fig. 12: Relative abundance of zooplankton at the Tomaro creek (October, 2007 to March, 2008).

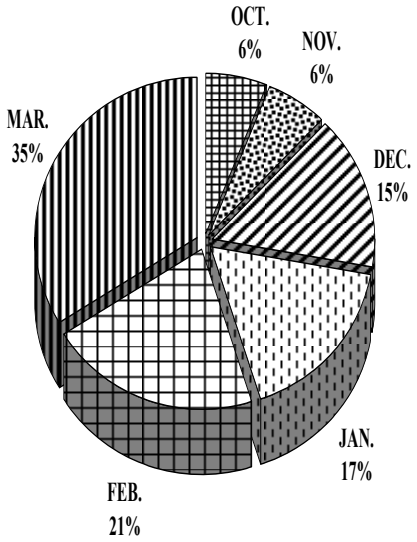

Fig. 14: Monthly distribution and abundance of Zooplankton at the Tomaro creek (October, 2007 and March, 2008).

Community Structure Indices

The indices of Species richness (d), Shannon – Wiener (Hs), Menhinick (D), Evenness (j), and Simpsons' Dominance (C) were calculated for the monthly variation in zooplankton abundance and composition and are presented in Table 3. The Margalef's index was highest (1.75) in February and lowest (0.26) in both October and November, the Shannon - Weiner index was lowest (0.22) in November and highest (0.85) in February. Species richness value ranges between 0.26 and 1.75mg/L. The highest value of 1.75mg/L was recorded in February and the lowest value 0.26mg/L was recorded in October and November, while the average value was 0.89. The diversity index value ranges between 0.22 to 0.85mg/L. The highest value (0.85mg/L) was recorded in February and the lowest value 0.22mg/L was recorded in November, while the average value was 0.55. The evenness / equitability values ranged between 0.72 and 0.97mg/L. The highest value (0.97mg/L) was recorded in October and the lowest value 0.72mg/L was recorded in November, while the average value was 0.83. The Simpsons' Dominance Index value range between 0.06 and 0.68mg/L. The highest value (0.68mg/L) was recorded in November and the lowest value (0.06mg/L) was recorded in February.

Table 3: Zooplankton community composition parameters at the Tomaro creek (October, 2007 to March, 2008).

PARAMETERS	MONTHS							
	OCT.	NOV.	DEC.	JAN.	FEB.	MAR.	Mean	Std. Dev.
Total species diversity (S)	2	2	4	7	10	8	5.5	±3.33
Total zooplankton abundance (N)	50	50	125	140	170	275	135	±84.14
Log of Species diversity (Log S)	0.3	0.3	0.6	0.85	1	0.9	0.66	±0.31
Log of zooplankton abundance (Log N)	1.7	1.7	2.1	2.15	2.23	2.44	2.05	±0.29
Shannon-Wiener Index (Hs)	0.29	0.22	0.47	0.72	0.85	0.74	0.55	±0.26
Menhinick Index (D)	0.28	0.28	0.36	0.59	0.77	0.48	0.46	±0.19
Margalef Index (d)	0.26	0.26	0.62	1.21	1.75	1.25	0.89	±0.61
Equitability Index (j)	0.97	0.72	0.78	0.85	0.85	0.82	0.83	±0.08
Simpson's Dominance Index (C)	0.52	0.68	0.4	0.24	0.06	0.22	0.35	±0.22

TABLE 5: SPEARMAN'S CORRELATION COEFFICIENT ASSOCIATIONS FOR SPECIES ABUNDANCE, RICHNESS AND CHLOROPHYLL *a* AT THE TOMARO CREEK (October, 2007 to March, 2008).

	PARAMETERS	CORRELATED		
		ZOOPLANKTON ABUNDANCE (N)	TOTAL SPECIES DIVERSITY (S)	CHLOROPHYLL-*a* (mg/L)
1	Air Temperature (°C)	✓ (-)	✓ (-)	X
2	Water Temperature (°C)	✓ (+)	x	✓ (-)
3	Transparency (cm)	✓ (+)	✓ (+)	X
4	Total Dissolved Solid (mg/L)	✓ (+)	✓ (+)	X
5	Total Suspended Solid (mg/L)	X	✓ (+)	✓ (+)
6	pH	✓ (+)	✓ (+)	✓ (+)
7	Acidity (mg/L)	✓ (+)	✓ (+)	X
8	Alkalinity (mg/L)	✓ (+)	✓ (+)	X
9	Salinity (‰)	✓ (+)	✓ (+)	X
10	Chloride (mg/L)	✓ (+)	✓ (+)	X
11	Conductivity (µS/cm)	✓ (+)	✓ (+)	X
12	Dissolved Oxygen (mg/L)	✓ (+)	✓ (+)	X
13	Biochemical Oxygen Demand (mg/L)	✓ (+)	✓ (-)	✓ (-)
14	Total Hardness (mg/L)	✓ (+)	✓ (+)	X
15	Calcium (mg/L)	✓ (+)	✓ (+)	X
16	Magnesium (mg/L)	✓ (+)	✓ (+)	X

17	Zinc (mg/L)	✓ (+)	X	✓ (-)
18	Iron (mg/L)	✓ (+)	X	X
19	Copper (mg/L)	✓ (+)	✓ (+)	X
20	Nitrate (mg/L)	X	✓ (+)	X
21	Sulphate (mg/L)	✓ (+)	✓ (+)	X
22	Phosphate (mg/L)	X	X	✓ (+)
23	Silica (mg/L)	✓ (+)	✓ (+)	X
24	Chlorophyll *a* (µg/L)	X	X	1
25	Species richness (S)	X	1	X
26	Zooplankton abundance (N)	1	✓ (+)	X

Key:

✓ (+) → Strongly positive (≥ ±0.40)
✓ (-)→ Strongly negative (≤ ±0.40)
x → not strongly correlated

DISCUSSION

The Tomaro creek of South-western Nigeria form part of the numerous ecological niches associated with the Nigerian coastal environment and according to Nwankwo and Akinsoji (1992), these creek and lagoons are linked to the sea through the Lagos Harbour which remains open all through the year. The variation in the water quality indices of the Tomaro creek during the course of the investigation could be attributed to the effect of tidal sea water incursion and, also freshwater input from adjoining rivers and creeks.

Air temperature was high throughout the study period, with the highest value (31^0C) recorded in December. The present observation also revealed a high surface water temperature all through the sampling period. Water temperature is usually high in the tropics. Transparency was observed to increase progressively with the dry season. These confirm the phenomenon that transparency and rainfall are inversely related, with increase in rainfall leading to a decrease in the transparency of the creek water. Furthermore, Nwankwo (1990) highlighted that seasonal variation in transparency in the coastal waters of South-western Nigeria is linked to the rainfall pattern and associated floods. According to Olaniyan (1969), pH is an indicator of environmental condition and the result of chemical condition in an aquatic environment. The study site was alkaline through out the study. According to Nwankwo (1984), high pH values observed in coastal waters of Nigeria may be due to the buffering effects of the sea water.

Salinity which creates horizontal environmental barriers to the Lagos lagoon biota is directly linked to the rainfall pattern (Nwankwo, 1996, 1998). The continued increase in salinity values observed during the investigation was likely due to increased incursion of tidal seawater, coupled with increase in evaporation rate. According to Barnes (1980), the complex variation of surface water salinity in lagoons is not determined by the alteration of wet and dry seasons, but by the nature of seawater and freshwater inflow coupled with the occurrence of strong winds. Barnes (1980) also highlighted the fact that in the wet seasons, lagoons are diluted considerably by freshwater from rain and river systems, while in the dry season, evaporation becomes more prominent. Salinity regimes in the Lagos lagoon have also been related to rainfall distribution (Nwankwo and Amuda, 1993).

It follows that salinity values were low in the creek because of the freshwater discharge from the adjoining wetland through creeklets (Nwankwo, 1991) during the rains. Furthermore, in the dry months, tidal incursion was well noticed inland which raised the salinity of the water considerably. This confirms a report by Olaniyan (1969). However, in October 2007, there was a decrease in the salinity values at the study site possibly due to the effect of rainfall and consequently, increase in freshwater inflow. According Fagade and Olaniyan (1974) and Nwankwo (1996), salinity is an environmental barrier in the distribution of biota. The intrusion of tidal seawater hinterland makes salinity an important factor in the creek (Nwankwo and Amuda, 1993). According to Onyema and Emmanuel (2009) evaporative concentration and reduced or minimal floodwater/river inputs encouraged increases in salinity, chloride, total dissolved solids, total hardness, conductivity and cations values among others in the dry season for the Lekki lagoon.

Conductivity increased with rise in the total dissolved solids and a decrease in the total suspended solids and increases during the dry months. The chloride values observed increased appreciably monthly while the acidity and alkalinity values in the study site showed notable monthly values in October and March. The continued increase in the total hardness values could be

due to increase in conductivity values throughout the sampling period. The Calcium and Magnesium concentrations were also high throughout the period of study. The heavy metal concentrations of the study site were low and showed monthly variations throughout the study period.

The nutrient level recorded during the period of investigation was high for sulphate. Most tropical waters have low nutrients values, a feature considered common for natural and polluted waters, but the level of sulphates and nitrates recorded during the study is suggestive of both chemical and organic pollution and nutrient enrichment. The high levels of nitrate-nitrogen and sulphide-sulphur may be due to the effect of direct discharges of pollutants such as municipal sewages and other biodegradable wastes into the coastal waters coupled with the enrichment of adjoining wetlands, creek and subsequent run-offs. This corresponds with the observation of Nwankwo (1993) for the coastal water of south-western Nigeria. Also, Nwankwo (1995) is of the view that storm water channels, creek and creeklets acts as conduits for land based human-induced activities into the coastal waters. The phosphate-phosphorus concentrations were very low throughout the sampling period. Furthermore, according to Nwankwo (1984) and Nwankwo and Akinsoji (1992), rainfall also introduces chelating agents as well as increase the nutrient levels of the lagoons. The silica concentration of the study site was observed to increase appreciably but the values were recorded in the rainy months. According to Nwankwo (1998), Dissolved oxygen decreases with increased temperature and Biochemical Oxygen Demand due to increase metabolic activities of most species probably inducing bacteria and fungi which are common in polluted sites in the Lagos lagoon (Akpata and Ekundayo, 1983) but the relatively high Biochemical Oxygen Demand (BOD) values and the relatively high Dissolved Oxygen (DO) values observed through the investigation may be to the photosynthesis activities which released oxygen.

Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) can be used to determine the level of pollution in a water sample. With regard to the COD values were relatively high, probably indicating a high level of pollution within the creek.

The abundance and diversity of species similarly varied with rainfall pattern. More zooplankton taxa were observed in February and March. This may probably be due to the increased rainfall volume in these months which consequently led to an increase in flood effects, a resultant dilution of the water body and an increase in the population of freshwater species. The present observation showed that the number of individuals of the zooplankton species decreased with an increase in the amount of rainfall throughout the period of investigation. This however, corresponds with Hill and Webb (1958), Olaniyan (1969) and Nwankwo (1991, 1993; Onyema, 2003, 2007, 2008) who reported that floods associated with rainfall dilutes the ionic concentration of the coastal waters and breaks down any horizontal, environmental gradient within the lagoon system. However, there was an increase in the abundance and species diversity of copepods and some juvenile stages in February and March. Despite the sharp increase in the salinity of the water, which probably maybe due to the high tolerance of these zooplankton species to high salinity environments. Data on the Species richness index and Diversity index, Equitability and the Simpson Dominance index showed monthly variation in conformity with the zooplankton distribution. High values for the diversity index indicate that the species were more evenly dispersed. The dominance by more species was attributed by very high species diversity and high species richness. Hence it follows that rainfall and salinity are known to regulate the occurrence and distribution of biota in the Tomaro creek. This corresponds to the findings of Olaniyan (1975), Hill and Webb (1958) and Nwankwo (1990) that two physiological factors, rainfall and salinity determine the hydro-climate of the coastal lagoons of South-western Nigeria. Also, it was observed that zooplankton abundance and salinity of the Tomaro creek showed a strong positive correlation ($r = 0.87$).

From the present observations, it is possible that rainfall affects other environmental factors. This confirms the reports from Webb (1960) that rainfall in the tropics is more important than temperature in determining environments. From the observations Biochemical Oxygen Demand ($r= -0.40$) show a strongly negative correlation while salinity ($r= 0.87$) and Dissolved Oxygen ($r= 0.62$) showed a strongly positive correlation. Conductivity and Total Dissolved Solids also showed a strong positive correlation. While zooplankton abundance and water temperature ($r = 0.45$) show a strong positive correlation, indicating that an increase in rainfall consequently lead to decrease in the water temperature.

Arthropods were the most abundant and more diverse group observed while some unidentified jelly-fish, a Cnidarian were frequent individual species observed during the investigation. However, various juvenile stages were also observed, with the nauplii larval stages the more abundant of the stages, having a total number of 95 individuals. Among the Arthropods, *Acartia discaudata, Acartia clausii, Acartia tonsa* (Giesbrecht), *Calanus finmarchicus* (Gunn.), *Centropages furcatus* (Dana) and *Paracalanus parvus* Claus were prevalent.

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Table 5: Spearman's Correlation coefficient matrix of Water Quality Indices, Chlorophyll-*a* and Zooplankton diversity and Abundance at the Tomaro creek from October, 2007 to March, 2008.

	Air temp.	Water temp.	Transparency	Rainfall	TDS	TSS	pH	Acidity	Alkalinity	Salinity	Chloride	Conductivity	DO	BOD	COD	Total Hardness	Calcium	Magnesium	Zinc	Iron	Copper	Nitrate	Sulphate	Phosphate	Silica	Chlorophyll – *a*	Species diversity (S)	Species abundance (N)
Air temp.	1																											
Water temp.	0.20	1																										
Transparency	-0.73	-0.26	1																									
Rainfall	0.45	0.33	-0.66	1																								
TDS	-0.75	-0.03	0.95	-0.85	1																							
TSS	-0.59	0.6	0.79	-0.18	0.59	1																						
pH	-0.78	-0.37	0.75	-0.9	0.91	0.38	1																					
Acidity	-0.43	0.17	0.89	-0.81	0.91	0.48	0.70	1																				
Alkalinity	-0.76	0.18	0.88	-0.54	0.85	0.59	0.68	0.78	1																			
Salinity	-0.71	-0.01	0.94	-0.85	0.99	0.55	0.88	0.94	0.87	1																		
Chloride	-0.73	0	0.94	-0.82	0.99	0.56	0.87	0.93	0.99	0.99	1																	
Conductivity	-0.69	0.03	0.95	-0.84	0.99	0.58	0.86	0.95	0.86	0.99	0.99	1																
DO	-0.8	-0.17	0.64	-0.4	0.67	0.29	0.6	0..54	0.88	0.7	0.75	0.67	1															
BOD	0.24	0.03	-0.47	0.78	-0.59	-0.24	-0.69	-0.56	-0.39	-0.58	-0.57	-0.58	-0.1	1														
COD	0.65	0.04	-0.38	0.25	-0.38	-0.57	-0.56	-0.03	-0.29	-0.29	-0.29	-0.29	-0.16	0.51	1													
Total Hardness	-0.08	-0.10	0.96	-0.70	0.95	0.64	0.82	0.87	0.97	0.96	0.98	0.96	0.81	-0.5	-0.36	1												
Calcium	-0.86	0.05	0.87	-0.58	0.87	0.59	0.78	0.73	0.98	0.88	0.91	0.86	0.89	-0.45	-0.44	0.97	1											
Magnesium	-0.36	-0.05	0.81	-0.82	0.86	0.44	0.68	0.93	0.56	0.86	0.82	0.88	0.33	-0.48	-0.01	0.72	0.53	1										
Zinc	-0.03	0.79	0.58	-0.08	0.38	0.52	-0.02	0.63	0.6	0.44	0.46	0.46	0.31	-0.09	0.26	0.51	0.44	0.44	1									
Iron	-0.18	0.78	0.63	-0.15	0.42	0.88	0.16	0.47	0.34	0.4	0.38	0.44	-0.09	-0.31	-0.34	0.41	0.28	0.49	0.62	1								
Copper	-0.8	-0.21	0.69	-0.68	0.79	0.36	0.86	0.6	0.83	0.78	0.82	0.76	0.78	-0.66	-0.57	0.84	0.91	0.39	0.14	0.07	1							
Nitrate	0.19	0.34	-0.17	-0.49	-0.34	0..34	-0.42	-0.39	-0.41	-0.41	-0.44	-0.37	-0.59	0.47	-0.09	-0.4	-0.5	-0.13	-0.13	0.45	-0.67	1						
Sulphate	-0.07	0.13	0.96	-0.72	0.96	0.62	0.8	0.91	0.97	0.97	0.98	0.97	0.78	-0.53	-0.3	0.99	0.95	0.76	0.57	0.43	0.82	-0.45	1					
Phosphate	-0.19	-0.58	-0.02	-0.31	0.13	-0.03	0.35	-0.09	0.05	0.05	0	-0.06	-0.25	-0.03	-0.29	-0.1	-0.21	0.25	-0.68	-0.07	-0.11	-0.43	-0.15	1				
Silica	-0.06	-0.12	0.35	-0.79	0.5	0.12	0.62	0.51	0.49	0.49	0.45	0.49	-0.14	-0.93	-0.39	0.32	0.2	0.55	-0.05	0.29	0.43	-0.28	0.36	0.29	1			
Chlorophyll *a*	0.09	-0.65	-0.08	-0.65	0.19	-0.37	0.45	0.14	0.16	0.16	0.11	0.15	-0.28	-0.54	-0.13	-0.08	-0.18	0.30	-0.56	-0.24	0.11	-0.17	-0.05	0.68	0.11	1		
Species diversity	-0.79	-0.15	0.9	-0.85	0.98	0.49	0.93	0.87	0.98	0.98	0.99	0.97	0.77	-0.56	-0.35	0.96	0.9	0.79	0.31	0.26	0.84	-0.46	0.95	0.11	0.84	0.19	1	
Species abundance	-0.65	0.45	0.97	-0.51	0.86	0.84	0.6	0.84	0.87	0.87	0.88	0.88	0.62	-0.40	-0.32	0.92	0.85	0.72	0.72	0.71	0.62	-0.13	0.93	-0.21	0.62	-0.27	0.80	1

Combating Climate Change in Kenya: Efforts, Challenges and Opportunities

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Abstract: Increase in emissions of CO_2 gas and other greenhouse gases (GHG) such as methane, nitrous oxide, CFC, HCFC and halogens into the atmosphere has led to the overall rise in mean global temperature over the years and the resultant climate change. Key anthropogenic activities responsible include fossil fuel combustion and land-use changes especially tropical deforestation. For developing countries such as Kenya, climate change is a threat to livelihood support systems. Kenya is currently experiencing the effect of climate change especially variation in weather patterns. Prolonged drought and famine has currently left over 10 million people faced with starvation, while floods and resurgence of pests and diseases have been noted in other parts of the country. Widespread poverty, inadequate socio-economic resources and a large climate-dependent agricultural sector makes the country vulnerable to the vagaries of climate change and ill-equipped to adapt to the long-term changes in climate. In spite of these, Kenya has embarked on various measures to mitigate climate change such as adoption of clean development mechanism, re/aforestation and spread of green technology. This research focused on Kenya's effort hitherto in combating climate change, the challenges thereon and opportunities for improvement. [Report and Opinion. 2009;1(6):65-76]. (ISSN: 1553-9873).

Key words: Climate change; Impacts; Adaptation; Mitigation; Kenya

1.0. Introduction

According to the International Energy Agency (IEA), world emissions of carbon dioxide (CO_2), the leading GHGs responsible for climate change, mainly emitted from fossil fuel combustion, has increased tremendously over the past 2 decades with a rise from 20.8giga tons in 1990 to 26.6 gigatons in 2004(IEA, 2006). IEA projections show that non-annex 1 countries (including Kenya) and other developing nations will soon surpass Annex 1 countries in CO_2 emissions with China contributing a quarter of this emission in the 2020s (IEA, 2006; World Bank, 2008). The Earth's mean global temperature has been rising steadily since the pre-industrial period before 1750 with a notable rise of 0.55^{o}C in the 1990s. 11 of the 12 hottest years on record occurred in the past decade (IPCC, 2007b).

Historical climate data show that the African continent is already undergoing climate change where temperature rose by 0.7^{o}C in the 20th century with a projected increase of between 0.2 to 0.5^{o}C degrees in the next decade (see Figure I). The changes have led to reduced precipitation in the Sahel and a net increase across Eastern and Central regions. Impacts of climate change will not be felt in the same magnitude across the globe. According to the Inter-governmental Panel on Climate Change's (IPCC) Regional Climate Change Index(RCCI), Africa ranks lower than Central America (which ranks highest) and the highlands of Central Asia (IPCC, 2001). {RCCI is the IPCC's comparative index designed to identify regions which will be most affected by climate change. It is based on regional mean precipitation range, mean surface air temperature and change in precipitation and temperature inter-annual variability (Ibid)}. Although Africa ranks lower, her impacts may be more widespread and severe due to widespread poverty; low infrastructure development; recurrent droughts, inequitable land distribution and overdependence on rain-fed agriculture making it highly vulnerable to the impacts of climate change. For Kenya, climate change is a threat to national development, community livelihood support mechanisms and a threat to environmental management thus combating climate change is indispensable.

1.2. Country Description

Kenya lies within co-ordinates 1 00 N and 38 00 E in East Africa. She borders Ethiopia to the North, Somalia to the East, the Indian Ocean to the South East, Tanzania to the South, Uganda to the West and Sudan to the North West. She has an area of 582,646km^2 of which 11,230km^2 is the water mass while 80% of

terrestrial land is arid and semi-arid. The population is 33.4 million people (Kenya, 2006).

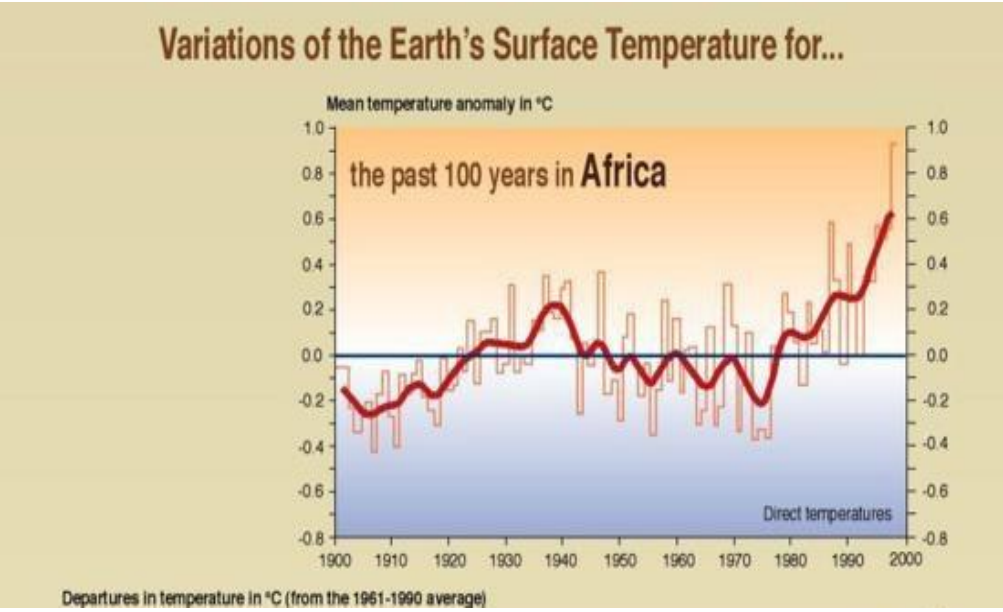

Figure I: Variations of the Earth's Surface Temperature for the past 100 years in Africa (Image adopted from WMO, UNEP, 2001)

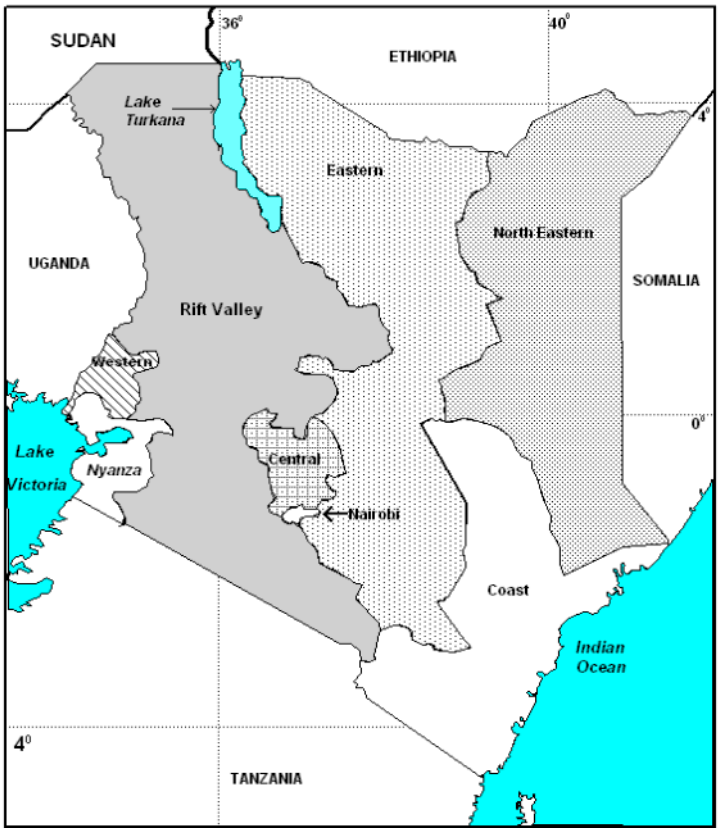

Figure II: Administrative map of Kenya showing provinces

1.2.1. Climate

Metrologically, Kenya lies in one of the most complex sectors of the African continent. Its climate is influenced by large-scale tropical controls which include several major convergence zones including the Inter-tropical Convergence Zone(ITCZ) that are superimposed upon regional factors associated with lakes, topography and the maritime influence. Thus, the climatic patterns within the country are markedly complex and change rapidly over short distances (Wandiga, 2006). Annual temperature range is 2 ^{o}C with the lowest value in March and April and the highest is in July and August. Diurnal temperature range is in the order of 10-20^{o}C, far exceeding the annual temperature range. Mean annual net radiation received on a horizontal surface is between 450-550 cals/cm^{2}/day. Mean annual bright sunshine amounts to over 7-8 hours per day in the highlands and 8-9 hours per day in the lowlands (Ibid).

Rainfall is distributed in short and long rainy seasons with the former received in October to December and the latter in April to June while July and August are the coldest months. Rainfall is influenced by conventional and relief microclimates depending on location. In addition, the rainfall variability is closely linked to the El-Niño Southern Oscillation (ENSO) phenomenon and the Sea-Surface Temperatures (SSTs) fluctuations in the equatorial Indian and Atlantic Oceans. The rains are normally enhanced during the ENSO years which occur every 5 to 6 years. Thus, such high climate variability is likely to enhance due to climate change in turn enhancing climate change impacts both at regional and local scales.

2.0. Anthropogenic impacts contributing to climate change in Kenya

Major impacts contributing to climate change in Kenya include:- overutilization and degradation of natural resources; reduction in tree cover on farmlands, soil erosion and deforestation; industrialization; rapid urbanization with a projected 60 percent of total population bound to live in the cities by the year 2030(Kenya, 2007); and all foregone are driven by rapid population increase. This paper will expound the impact on the deforestation and wetland degradation since their conservation and management acts as major opportunities for carbon sinks.

2.1. Wetland and water resource degradation

Wetlands are defined as areas of marsh, fern, peatlands or water whether natural or artificial, permanent or temporary with water that is static or flowing, fresh, brackish or salty, including areas of marine water the depth of which at low-tide does not exceed six meters (http://www.ramsar.org/cda/ramsar/display/main/main.jsp?zn=ramsar&cp=1_4000_0). Wetlands play an important role in the global carbon cycle and contribute 15 percent of total terrestrial carbon storage (Zhou, et al, 2007). However, the ability of wetlands to act as carbon reservoirs depends on their management state since they can also act as carbon sources by emitting carbon dioxide and methane into the atmosphere. Wetlands are highly dynamic ecosystems, changing with seasons and over long periods of time. They are also influenced by factors that lie far beyond their boundaries since they transcend private, communal and public property regimes where property management may enhance or undermine the state of the wetlands. Thus, the major impact on wetlands as a sink of carbon is anthropogenic, where changes in land use and agricultural drainage affect the hydrological regime of the wetlands in-turn affecting their ability to sequester carbon.

In Kenya, wetlands are additionally defined as areas of land that are permanently or occasionally water-logged with fresh, saline, brackish or marine waters, including both natural and manmade areas that support characteristic biota (Kenya, 2005). Such areas cover about 3-6% of the Kenyan land surface and they are irregularly distributed countrywide. Major wetlands lie within the major lakes and along the Coast. Kenya has 6 identified Ramsar sites of International Importance namely Lake Nakuru, Lake Baringo, Lake Bogoria, Lake Naivasha and Lake Elementiata, all in the Rift Valley province (NEMA, 2006). There are also hundreds of small wetlands such as swamps, small lakes, soaks dams and riverine flood plains that are distributed throughout the western and central Kenya highlands. In addition, of more valuable significance are the small wetlands that occur in the drier Arid and Semi Arid Lands (ASALs) as a result of occasional flows of ephemeral rivers and the output of springs from distant water sources. These are a lifeline to the people, livestock, wildlife and other biodiversity as a refuge in times of drought in such areas.

However, as development progresses, there is a rising dire threat to water resources especially freshwater wetlands. These are faced with pollution, drainage, encroachment, misuse, overexploitation and the threat of extinction. Coupled with these anthropogenic challenges are issues of policy and regulation in Kenya where the utilization and management of wetlands is sectoral scattered in various legislature. The Wetlands Sessional Paper is yet to be in effect. Alongside these is climate change, with the most visible impact globally, regionally and locally being the alternation of the hydrological regimes, affecting water

supply, quantity and increasing vulnerability among the poor. In addition, by virtue of their tropical location and hydrological regimes, shallow lakes and wetlands of Kenya are more susceptible to the damaging influence of oxygen-demanding pollution which is bound to rise with increase in temperature due to climate change hence their catastrophic degradation is imminent (Kipkemboi, et, al; 2007).

2.2. Deforestation and loss of tree cover on farmlands

Rapid deforestation and land use change of the world's forests especially tropical rain and dry-land forests contribute 15 percent towards global warming and the resultant climate change. Kenya's forest cover now stands at 1.7 percent, way below the recommended minimum of 10 percent (Kenya, 2007). Her main forests and water towers are The Aberdares Ranges, Mount Kenya Forest, Mount Elgon, Mau Forest Complex and The Cherenganyi Hills. Other forests include dry-land forests, community forests, local authority forests and coastal forests including the Coastal Mangrove and the *Kaya* Forests. The country has indigenous forests which are found in all the afore-mentioned forests and also plantation forests. Some of the indigenous forests such as the Mau Forest Complex belong to the great African tropical rainforests that stretch to the Democratic Republic of Congo. Plantation forests are mainly found in part of the Mau Forest Complex and the Nyayo Tea Zones.

High loss of forest cover in the country occurred in the last one and a half decades that were characterized by legal and illegal expansion of human settlements into gazetted forest lands; forest excision; expansion of farmlands due to high population pressure and unsustainable extraction of timber and non-timber forest products. Weak land-use and forest policies and regulations; corrupt practices and macro-economic policies that favored cash crop production for export – (tea and pyrethrum) are also to blame. Abuse of the *Shamba* system is also largely blamed for deforestation in Kenya.

The *shamba* system is a plantation forest management method in Kenya which is an adaptation of the *Taungya* system of South America and the Plantation System of Myanmar. This system allows communities surrounding a protected gazetted forest to plant short-season food crops within the forest's patches while at the same time planting and maintaining trees within the patches. When the trees mature (usually 3-5years), they shift to other patches and repeat the cycle. The community is not paid any wage for offering labor in tree planting and maintenance but they benefit by planting food-crops for their households. Poor implementation and enforcement of this system led to massive deforestation of protected forests and illegal settlement of communities within forest patches. The government banned the *Shamba* system in 1986 but it has now been re-introduced in the Forest Act of 2005.

Hitherto, logging and human settlement within the Mau Forest complex has resulted in reduction of water volume and/or drying up of key feeder rivers which drain into the Rift Valley Lakes – Nakuru, Baringo, Naivasha, Natron and Bogoria plus Lake Victoria in western Kenya threatening livelihoods and ecosystems far beyond the Kenyan boundary. Degradation of the Mau, Cherenganyi Hills and Mt Elgon Forests has resulted in annual flooding of regions around River Nzoia especially in Budalangi District.

Within the farm lands, loss of tree cover is attributed to land fragmentation for human settlement and agriculture, high demand for timber for the construction industry and wood-fuel for industrial use. In addition to these is the increasingly high demand for arable land to plant bio-fuel-generating crops in plantations to meet the global demand. In addition, 85 percent of domestic energy in Kenya is woodfuel where charcoal meets 80 percent of urban needs while in rural areas over 90 percent of energy is from firewood (Mugo and Ong, 2006). This is complicated with the fact that regulating the production and sale of charcoal which has been slow hence the trade has now become unsustainable especially in the ASALs where it has greatly contributed to loss of land cover and land degradation. In fact, the demand for woodfuel currently outstrips supply (Gichu, 2008). The new Forest Act of 2005 now addresses issues concerning charcoal trade but its implementation depends on proper enforcement and public education.

2.3. Impacts of climate change in Kenya

Africa is one of the most vulnerable continents to climate change - a situation aggravated by the interaction of multiple stresses occurring at various levels and as a result of its low adaptive capacity (IPCC, 2007a). Kenya is already experiencing the impacts of climate change and more are anticipated to occur with increased warming of temperatures. Direct impacts include changes in weather patterns with decreased rainfall, increased temperatures and higher evaporation rates in the dry areas. Under conservative warming estimates, rainfall is expected to increase by 5 to 20 percent during the months of December to February while a decrease of between 5 to 10 percent will occur in June to August. Under more rapid warming scenarios, Kenya and other East African

countries may receive up to double precipitation while arid areas are likely to receive even less than at present (IPCCC, 2001).

Indirect impacts of climate change concern socio-developmental strategies such as health, livelihood support, education and conflict. Frequent drought spells over the years have led to severe water shortage, increased risk of food shortage and expansion of aridity and desertification into marginal lands and changes in the planting dates of annual crops. There will be notable increase of fungal outbreaks and insect manifestations due to changes in temperature and humidity along with reduction in ecosystem integrity, its resilience and decline in biodiversity.

Other impacts include increase in human, crop and animal vector-borne diseases such as malaria, cholera and Rift Valley Fever; sea level rise resulting to inundation of low-lying areas along the coast and islands while increase in ocean acidity will result in coral reef bleaching along the Kenyan Coast. Melting of glaciers on Mount Kenya is already occurring while extreme weather events will increase; *inter alia* (Case, 2006; Githeko, et al, 2000; IPCC, 2007a; NEMA, 2008; Orindi and Murray, 2005; UN, 2001; Wandiga, 2006). Loss of biodiversity, spread of disease margins and inundation of low-lying coastal areas will severely affect the tourism sector which is Kenya's second foreign exchange earner.

2.3.1. Water availability

Kenya is a water scarce country with over 80 percent of the total land area regarded as ASAL. The country has a freshwater per capita of $647m^3$ against the United Nations' recommended minimum of $1,000m^3$ with a projected decline to $235m^3$ by 2025 unless effective measures are implemented to address the challenge (Kenya, 2007). Water abstraction rate is only 5.5 percent of which 84.7 percent is surface water and the rest is groundwater. Surface waters are threatened with pollution from industrial and domestic sources as well as high sediment load from farmlands due to soil erosion. Climate change is predicted to cause changes in the frequency, intensity and unpredictability of precipitation with adverse effects on water availability, agricultural production, health and widespread food shortages (Case, 2006). Reduction in water availability will in-turn affect all sectors of development especially agriculture which is mainly rain-fed and health of which 80 percent of Kenya's illnesses are water-related (Kenya, 2007). In the rural areas, water scarcity will increase conflict since all economic and social activities have a water dimension. This is especially so among the pastoral and nomadic communities.

2.3.2. Impact on development and the economy

The economic sector is vulnerable to climate change sensitivity with huge economic impacts. This vulnerability is exacerbated by existing developmental challenges of endemic poverty; complex governance and institutional dimensions; limited access to capital including markets; inadequate infrastructural and technological development; ecosystem degradation and complex natural disasters (IPCC, 2007a). In Kenya, the economic sector is mainly agriculture-driven thus sensitive to change in this sector. Climate change is/will negatively affecting/affect food production especially tea production in the Kenya Highlands which is sensitive to temperature and rainfall in turn affecting income from the leading foreign exchange earner.

2.3.3. Melting of glaciers on Mt Kenya

The Mountain's glaciers are retreating rapidly where, in 1990, there were 18 glaciers on the mountain, now only 7 are left (Kenya, 2007). This is attributed both to climate change and seasonal aridity within the Mountain microclimate. This in turn has affected the water levels in Tana and Athi Rivers among several rivers that originate from this mountain. Tana and Athi Rivers are the main sources of water for the Seven Folk Dams, Kenya's principal hydroelectric power generation stations. Tana River, the longest river in Kenya is 650 kilometres long and has a catchment area of 94,700 square kilometres while Athi has smaller catchment of 38,000 square kilometres (Rowntree, 1990). Originating from the southeastern slopes of Mt. Kenya, they flow eastwards towards their mouth in the Indian Ocean. These rivers encompass all or parts of over 20 districts within 5 provinces of North Eastern, Eastern, Central, Rift Valley and the Coast. Reduction in water volume of these two rivers due glacier disappearance on Mount Kenya as a result of to climate change will threaten the lives of over half of the Kenyan population.

2.3.4. Extreme events and natural disasters

Over 70 percent of natural disasters in Kenya are weather-related and their frequency has increased over the years with drought and floods being the main disasters. The drought oscillation period in past years recurred every 5 years but has now reduced to every 2 to 3 years. The worst drought since independence was in 1991-92 while the 1997-2000 was the worst in the past 40 years. During the latter drought, nomadic communities incurred over 50 percent losses in livestock while food shortages were felt countrywide.

Currently (2008/09), Kenya is facing one of the worst droughts that has left over 10 million people without food and access to drinking water. This disaster

has been exacerbated by a combination of other factors including the 2007/8 post-election violence that affected agricultural production in Kenya's bread basket areas, current global economic recession that has affected the purchasing power of the people, inadequate disaster response mechanism which is often disaster response rather than prevention and other governance issues.

The worst floods in Kenya occurred in 1997/98 El-Nino rains that resulted in displacement of persons, damage of physical infrastructure such as roads, bridges and railways, spread of human and livestock diseases *viz* cholera, malaria and Rift Valley diseases respectively, damage to agricultural produce and economic fiscal inflation (Kenya, 1998). Climate change is predicted to enhance drought, flood, fire, ENSO and tropical storms within the country and along the Coastal zone.

2.3.5. Health

Various vector-borne human and animal diseases are likely to rise with increase in climate change. These include malaria, dysentery and cholera in humans and Rift Valley Fever in livestock. Kenya has an infant mortality rate of 77 deaths per 1,000 while the under five years old mortality rate lies at 115 deaths per 1000. The total fertility rate is 77 with a 1:1.1 male to female ratio (Kenya, 2006). Changes in climate are bound to affect the health of the nation increasing morbidity, mortality and general vulnerability to other vagaries of nature.

Malaria: More than 90 percent of global mortality due to malaria occurs in Africa accounting for up to 1 million deaths per annum. In Kenya, Malaria is the leading cause of morbidity and mortality in children and adults, responsible for over 40,000 infant deaths per annum (Wandiga, 2006). The disease is endemic in Kenyan lowlands but is unstable in the highlands where, before the 1980s, highland malaria was intermittent and was mainly triggered by sensitive climate variability and environmental change. However, in the last two decades, resurgence of highland malaria epidemics has been largely associated with climate change, socio-economic change, deterioration of health care and food production systems, poverty and modification of microbial/vector adaptation.

Land clearance in the highlands for human settlement due to population pressure has led to elevated temperature in the highlands and provided ideal vector-breeding grounds. Research (Githeko and Ndegwa, 2001) indicates that the prevalence of highland malaria is differential by elevation with 70%, 40% and 30% in valley bottom, hillside and hilltop respectively for those living 1,100m above sea level. With climate change, the malaria belt is gradually moving towards higher altitudes threatening the lives of highland dwellers most of whom have not developed resistance to the disease.

3.0 Efforts made towards combating climate change in Kenya

Ratification of the Kyoto Protocol: Kenya ratified the Kyoto Protocol of the United Nations Framework Convention on Climate Change (UNFCCC) in August 1994 and has over the years actively participated in and hosted the Conference of Parties. The country has also operationalised the implementation of target objectives and agreements albeit at lower scale due to socio-economic challenges. The Government has also set modalities for participation in the Clean Development Mechanism (CDM), one of the flexible mechanisms of article 12 of the Kyoto Protocol that allows industrialized countries (Annex I countries) to finance investment projects for greenhouse gas emission reduction in developing countries so as to generate credits which can be used to meet their own commitment to the Protocol. In this regard, relevant ministries and state corporations in Kenya are in charge of major components of the CDM through licensing, inspection, monitoring and approval of related projects. Thus, 1) National Environment Management Authority is the Designated National Authority of the CDM; 2) Kenya Forest Department is in charge of all CDM-re/aforestation and agroforestry projects; and 3) the Ministry of Energy is in charge of all energy conservation and alternative energy projects.

3.1. CDM Projects in Kenya

3.1.1. Kenya Electricity Generating Company (KenGen) CDM projects

KenGen, Kenya's main electricity generating company is the leading organization in the country that has embarked on various CDM projects. Key among these are re-development of existing hydropower and gas-power projects to produce clean energy in turn reducing CO_2 emissions into the atmosphere and also participating in carbon trading within the CDM. In 2006, KenGen developed Project Idea Notes (PINs) for nine of its many power generating projects and submitted them to the World Bank, 6 of which were accepted for emission trading. As at 2008, Olkaria II Geothermal Project and Sondu Miriu Hydropower Project's Project Design Documents (PDDs) had been submitted to the UNFCCC website for public comments (Kollikho, 2009).

By participating in CDM, KenGen will earn approximately USD 6.5 million per annum from the World Bank up to the year 2012. In addition, most of these projects will earn an additional USD 1 per ton of

CO_2 from the carbon fund as direct benefit to the communities surrounding the projects. After 2012, that is, after the expiry of phase 1 commitment period of the Kyoto Protocol, the World Bank will purchase the carbon emissions of these 6 projects as Verified Emission Reductions (VERs) at a reduced price of USD 2 (Ibid). By participating in CDM, KenGen is enhancing the production of clean energy while at the same time increasing the cumulative number of clean energy projects in the country. KenGen is also involved in water catchment conservation in all the 5 water towers of Kenya through a tree planting partnership with local communities under the Kenya Sector Environment Program.

Project	**CER per ton of CO_2 to be paid to KenGen by World Bank(US$)**
Tana Redevelopment Hydropower Project	13.9
Kiambere Optimization Power Project	13.9
Kipevu Combined Cycle Plant	12.9
Olkaria II Geothermal Project	10.5
Eburru Geothermal Project	13.9
Sondu Miriu Hydro-Power Project	10.9

Table I: Kenya Electricity Generating Company's CDM projects accepted by the World Bank for Certified Emission Reduction (CER)

3.1.2. The International Small Group and Tree Planting Program (TIST)

Working in collaboration with Carbon Footprint Limited (United Kingdom-based on-line commercial enterprise trading in carbon offsets and offering consultancy services), TIST has a tree planting project based in Laikipia and Meru Districts, Central Kenya taking advantage of the Voluntary Emission Reductions(VERs). 1,415,715 trees have been planted by 2,650 small groups funded by USAID (Murray and Dey, 2009). The groups are closely monitored and trained which has enhanced the groups' capacity in participating in carbon sequestration projects and use of new technologies. The woodlots planted by the groups can be observed from Google map since the trees are planted in groves with clear GPS co-ordinates. Carbon offsets are sold to Carbon Footprint Limited which purchases them based on only the primary footprint with an estimate that 1 tree offsets approximately 730 kg of CO_2 in its full lifetime of 100 years. The offsets are then purchased on E-bay by online traders (Ibid). One challenge that this group has faced is high investments costs in establishing and running the project hence minimal benefits are trickling to the community.

3.1.3. Kiambu Community Group

This group is located on the Kikuyu Escarpment in Central Kenya and has an aforestation project that has hitherto planted over 300 hectares of trees and has been officially licensed by the Kenya Forest Service (KFS). Even though the group has not yet been officially registered as a CDM project due financial constrains, through KFS's support, it offloads carbon benefits to the Group through provision of tree seedlings and other community services. In addition, the group is selling carbon offsets to a British-based enterprise which is paying them for activities carried out thus far.

3.1.4. The Green Belt Movement (GBM)

GBM, the brainchild of Nobel Laureate Professor Wangari Mathaai, is an organization that has successfully conducted massive aforestation and reforestation in farmlands, community lands and schools in central and western Kenya and part of Eastern Africa by collaborating with women groups over the past two decades. Currently, GBM is still working with women groups in a CDM-Aforestation/Agroforestry project spread around Mount Kenya Region and the Aberdares Ranges. Hitherto, the groups have been able to collectively plant over 2,000 hectares of trees in pockets in the target area. Although they haven't yet been registered officially as a CDM project, registration is underway through the support of the World Bank. Thus far, the community is accessing some benefits from the World Bank that cover the cost of tree planting.

4.2. Renewable Energy Technology

Kenya has a high potential for utilizing renewable energy from biomass. Such energy is considered carbon neutral under the CDM since the CO_2 released into the atmosphere through biomass combustion is subsequently taken up by growing stock through the carbon cycle (Gichu, 2008). Various medium to large institutions such as schools, colleges, church-based mission centers, private and government organizations utilize solar and biogas energy as a cost-saving measure. These can be expanded countrywide to earn the additionality factor of CO_2 emission reduction that is necessary in climate change mitigation. At the same time, the rate of biomass extraction should match replacement. One such institution is the Kenya Tea Development Authority that has switched from using

crude oil to fuel wood for tea curing in most of its tea factories countrywide. The challenge however is that the switch to woodfuel was made prior to establishment of the tea companies' own tree plantations hence their usage of fuel wood from private suppliers is contributing to loss of tree cover on farm and community lands.

The country has also a vast potential for wind and solar energy if the cost of installation, technology transfer, and research and development in this area can be lowered in the global market. Currently, a 300MW wind power energy project in Lake Turkana has been constructed and will be commissioned in 2012. Funded by the African Development Bank, the project's output will contribute a quarter of the country's current electric power to the national grid – regarded as one of the largest wind energy projects in Africa. Solar power is widely used in industrial and domestic settings although installation the costs are prohibitive for widespread usage.

Biofuel: Jatropha species: The shrub is seen as one of the potential producers of biofuel that can be used as a substitute for diesel in industrial processing and transport. The species is well adapted to grow in harsh environments and can thus be widely grown in Kenya's unproductive and unutilized ASALs hence it will not interfere with food security while at the same time rehabilitating the degraded lands (Gichu, 2008). Magadi Soda Ash and Salt Mining Company have initiated a *Jatropha species* biofuel project in Magadi (Ibid). However, in-depth research is needed into the potential viability of the plant species versus its known negative characteristics such as toxicity of its leaves and nuts that necessitates careful handling by farmers and industrialists. It also has low productivity and is labor-intensive.

4.0. Challenges to combating climate change in Kenya

4.1. Economy

Kenya is a developing country whose growth model like many developing nations is based on the economic growth model that largely favors economic, industrial and physical development at the cost of environmental management. Thus, the current economic situation and state of the environment in Kenya has weakened the capacity of the country to adjust to the drastic economic and ecological changes are/will be induced and/or enhanced by climate change (Ominde and Juma, 1991). Coupled with this is the fact that over 80 percent of the population depends on agriculture as their livelihood. Hence, the challenge is how to share the insufficient economic resources to combat climate change and shift the overdependence of such a large populace from agriculture to alternative livelihood support mechanisms.

4.2. Poverty

Over 40 percent of the Kenyan population is food poor while another 30 percent is absolutely poor living on less than USD 1 per day(see figure III below showing the poverty head count(%) by province). The poor are often located in high-risk marginal areas and their lack of socio-economic resources means that they are ill-equipped to adjust to the long-term changes in climate. In addition, poverty undermines the health status of the people. Therefore, climate change will increase the poor's vulnerability and also reduce their adaptive capacity.

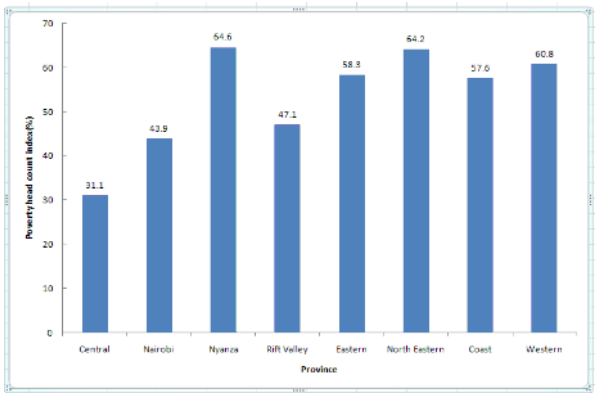

Figure III: Poverty head count index (percentage) by Province in Kenya (http://www.cbs.go.ke/downloads/pdf/Kenyafacts2006.)

4.3. Food insecurity

Demand for bio-fuels poses a threat to forest lands and arable land thereby increasing food insecurity. With increased GHG emissions, high energy costs of fossil fuel and energy insecurity, the world is turning to alternative sources of energy to meet their energy needs. Although the past two decades have seen increased progress in developing alternative energy, these technologies have not reached a level where they can replace conventional sources of energy.

5.0. Opportunities

5.1. Local adaptive capacity

Kenyan communities like many African communities have developed long traditions of social and community networks that have been used as livelihood coping strategies to climate changes and disasters for many centuries. Such strategies include switching to non-farm activities during dry spells, brick making, off-farm labor, transhumance, change in diet, shifting cultivation and

dependence of social networks and trust. Although such synergies are gradually weakening as a result of rural-urban migration, networks such as traditional land rights and systems of management are vital in reducing vulnerabilities of communities. Local adoptive mechanisms should be enhanced through capacity building and community support to enable communities utilize and manage natural resources in a sustainable manner in the face of climate change. Research (Orindi and Murray, 2005) suggests that understanding, documenting and strengthening of existing livelihood coping strategies rather than imposing new high-tech solutions will yield better results. However, other schools of thought (IPCC, 2007a) reckon that such adaptation options will cope with current changes for a while but will not be sufficient for future changes in climate.

Climate change adaptation strategies should be integrated into all levels of government so that climate change policies and development policies will not undermine but reinforce one another. Knowledge sharing through collaborative efforts between government, private sector, civil society and local communities will promote adaptation to climate change and enhance sustainable development. With regard to environmental management especially of natural resources, adaptation should be based on an ecosystem approach, which is a comprehensive and holistic approach to understanding and anticipating ecological change, assessing the full range of consequences and developing appropriate management responses. Existing disaster management mechanisms should be enhanced across all relevant sectors in addition to capacity building of target communities since the frequency and aggressiveness of the drought is bound to increase with climate change.

5.2. Policy

Kenya needs to formulate a comprehensive climate change policy to tackle climate change. The country is a signatory to several multilateral environmental treaties including the Agenda 21, Montreal Protocol, Basel Convention, Stockholm convention, Ramsar Convention , CITES and most importantly with regard to climate change, the Kyoto Protocol. Efficient and effective adoption and implementation of the various treaties and local policies will collectively contribute towards mitigating climate change.

5.3. Aforestation and re-forestation

Deforestation, soil erosion and loss of wetlands contribute 20 percent of the total anthropogenic carbon into the atmosphere. Forests and forest soils store vast amounts of carbon estimated to be 1 million tons, almost twice the amount found floating in the atmosphere. As critical components of the global carbon cycle, increase in forest cover in Kenya is thus a key way in enhancing carbon sequestration. As an equatorial country, her climate favors rapid growth of vegetation hence trees can sequester carbon from the air within a relatively short growth period (Gichu, 2008). In this regard, current efforts in forest conservation and management should be enhanced to include protection, restoration and sustainable use of forests and forest products. In well conserved and managed forests, trees can store up to 15 tons of CO_2 per hectare per year in their biomass and wood (Glenday, 2005). Forestry programs may aim for projects that can have the "additionality" factor stipulated in the CDM mechanism so as to gain from carbon trading and also increase the country's tree cover.

5.4. Voluntary Emission Reduction (VER)

VERs sprang up parallel to the CDM spearheaded by international nongovernmental organizations and other agencies in order to beat the bureaucracy in the CDM that has stringent rules and regulations that are often costly and time-consuming. The costs with the VER system are lower hence affordable to small scale projects. In Kenya, projects that can be accepted into the VER system include re-forestation programs within gazatted, local authority and community forests that have a canopy cover of more than 30 percent and do not qualify for the CDM under the Kenyan definition of a forest (Gichu, 2008).

5.5. Reduced Emission from Deforestation and Degradation in Developing Countries (REDD

This is an initiative which was discussed by the scientific and technical advisory body of the UNFCCC in May 2007 in Bonn, Germany. The program aims to reduce loss of forest cover and land degradation in developing countries in turn enhancing carbon sinks in soil and trees and cutting emissions from loss of forest cover and land degradation (Coomes, et al, 2008). Participating developing countries are rewarded for environmental services they provide such as environmental conservation, biodiversity protection, watershed management, carbon sequestration and landscape beauty at local and global levels.

According to ICRAF (http://www.icraf.net/downloads/publications/PDFs/PP08444.PDF), success of the REDD program hinges on taking a landscape approach to reducing carbon emissions and increasing carbon stocks through landscape management and sustainable agriculture. With the right policy incentives, mechanisms for

encouraging REDD could bring significant benefits to smallholder famers, to ecosystems and to the global climate. This is because it cuts the high costs of CDM - Aforestation project costs and is thus more likely to be adopted by low-income rural farmers. Kenya has been selected as one of the countries on which base-line survery for the REDD program will be based upon. Concerted effort in implementing the initiative will prove worthwhile in combating climate change.

Other opportunities include:-

- Investing in technologies on the supply side, such as clean electricity generation so as to increase efficiency and reduce carbon emissions.
- Carbon capture and geological storage for cement companies, petroleum refining factories and other large point sources of CO_2 may be an option in the near future although the technology is currently expensive. These include industries include Bamburi and East African Portland Cement Companies, Kenya Oil Refinery, *inter alia.*
- Carbon market: by participating in the carbon market, developing countries such as Kenya gain additional financial resources and cut their baseline carbon emissions by a big margin.
- Alternative livelihood sources that reduce pressure on the land resource base
- Lobby for user compensation in the global market for environmental services accrued by conserving the environment as an incentive for communities to participate in conservation and climate change mitigation.

Conclusion

Although developed countries will remain the largest per-capita emitters of greenhouse gases, the growth of carbon emissions in the next decades will come primarily from developing countries including Kenya. This is because these countries will be approaching their peak in industrial and economic development hence they will have a high energy utilization demand. That notwithstanding, climate change and its associated hazards will exert a heavy toll on Kenya threatening national social and economic development, environmental management and livelihoods support mechanisms. It will also widen the gap between the country's exposure to risks and her efforts to prepare for and manage them due to their frequency and intensity. The impacts will be felt more strongly on the poor especially in the ASAL regions of Kenya. The thrust therefore is to formulate adaptation and mitigation mechanisms tailor-made for the country that build up on existing adaptation strategies at all levels.

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Evaluating The Contribution Of Poultry Manure Application For Sustainable Crop Yield In Cassava (*Manihot Esculenta*)Based Cropping Systems.

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Abstract: The contribution of Poultry manure (10 t ha-^{1}) for sustainable crop yield in cassava based cropping systems (Cassava + Maize, Cassava + Melon, Sole Cassava etc), was evaluated for 5 years of continuous cropping in 2004, 2005, 2006, 2007 and 2008 growing seasons, at Imo State Polytechnic, Ohaji, Nigeria. A split – plot treatment arrangement fitted into Randomized Complete Block design with 3 replicates was used. The seed yield of melon in cassava / melon intercrop was progressively high at 10 t ha^{-1} of poultry manure application with peak (7.8 $^{-1}$) at the 4th and 5th year of planting while in maize + melon intercrop, and melon sole crop, the highest seed yields were recorded at the 3rd year of planting, 6.3 t ha $^{-1}$ and 6.5 t ha^{-1} respectively. The highest root yield (44.2 t ha^{-1}) of cassava was recorded at the 5th year of cropping in cassava/melon intercrop and 10 t ha^{-1} of poultry manure application but in cassava/maize system and sole crop cassava, the highest root yields, (25.8 t ha^{-1}) and(35.6 t ha^{-1}} were recorded at the 4th year of cropping. Irrespective of cropping systems, maize yield peaked at the 4th year of cropping. The highest LER (1.56)in cassava/melon mixture was recorded at the 5th year of cropping while that of cassava/maize (1.39) was recorded at the 4th year of cropping. Soil fertility was effectively sustained up to the 4th year of continuous cropping except in melon plots where it was maintained up to the 5th year with 10 t ha-^{1} of Poultry manure application. [Report and Opinion. 2009;1(6):77-86]. (ISSN: 1553-9873).

Key words: Intercropping, Poultry manure, sustainable crop yield.

Introduction

The use of poultry manure as an alternative to chemical fertilizer by rural farmers, in the South Eastern Nigeria is increasingly becoming popular because of non availability of conventional chemical fertilizers.

This popular choice of poultry manure over other organic manures may be associated with the ease involved in handling it. The manure is readily available and a bag of it (50kg) cost less than a dollar compared with the chemical fertilizer (N – P – K 20:10:10), a bag (50kg) of which cost about 4 dollars in the south eastern part of Nigeria.

Chemical analyses of the poultry manure showed that it contains the basic essential elements needed by crops in reasonable quantity more than some other organic manures and may thus form good alternative to compound chemical fertilizer, like N-P-K fertilizer, especially in the South Eastern Nigeria ,where the soil is heavily acidic (pH < 4.0) due to continuous cropping, improper and continuous application of chemical fertilizers. The use of inorganic fertilizer has been known to increase yam nematode population (*Obigbesan and Amalu 1985*), causes profuse weed growth (*Onochie 1975*) and nitrogen non-point pollution and acidification (*Agboola 1981*).

The superiority of Poultry manure over other organic manure has been confirmed *Hsich and Itsu (1993*) and Janadasa et al *(1997*) have reported that poultry manure application increased soil pH, organic matter, available phosphorus, exchangeable iron. *Djakoto and Stephen (1961)* observed that there was residue of “unexplained benefit” that occurred from the use of poultry manure which was probably connected with the balanced nutrient reserves slowly released during crop growth by the decomposition of the manure.

This unexplained benefit " of poultry manure which was connected with balanced nutrient reserves slowly released may lead to sustainable crop yield.

Poultry manure at much higher rates (10 – 50 tones/hectare) has been reported to give optimum response of marketable yield of *Corchorus olitorus* (*Adejoro 1999*), Cabbage (*Hochmuth et al 1993*), peppers, egg plants, tomatoes and Okra (*Maynard 1991*; *Hussein 1997 and Arotolu 1988.*

I. The present report forms parts of the series of experiments carried out to 1 assess the potential fertilizer value of poultry manure as lt affects the growth performance of the selected crops in cassava based cropping system,

ii. evaluate its effectiveness in sustaining the yields of the selected crops at a higher dose of application

iii. evaluate the incidence of weeds in respect of poultry manure application.

iv. determine its impact on soil physico-chemical properties and

v. assess the cost benefit ratio of each cropping systems as affected by poultry manure.

Materials And Methods

The field trials were conducted during the rainy (March - october) season of 2004, 2005, 2006, 2007, 2008, at the Research farm of Imo State Polytechnic, Umuagwo (Long. 07^{0}0E and Lat. 07^{0}07E). The soil of the experimental site is sandy loam in texture and contained pH 4.5 and organic carbon 0.51%. The soil N(g/kg), P(ppm) and K(cMol./Kg) were 0.31, 7.93 and 0.11 respectively. The region has a hot humid tropical climate and receives 1030mm ralnfall annually. A major part of the rain is received during April – October.

Randomized Complete Block Design (RCBD) with 3 replicates was used. The treatments, Poultry manure 0, 10 t ha^{-1} and Cropping systems (Cassava + Maize, Cassava + Melon, Sole Cassava, Sole maize and sole melon) were combined by split – plot treatment arrangement and fitted into the design used (RCBD). Thus a total of 10 treatment combinations as shown in Table I were used.

The Poultry manure was assigned to the main plots and the crop combinations assigned to the sub plots. Each block or replicate contained 10 plots, giving a total of 30 plots in the whole experiments. Each plot measured 3m x 4m separated from each other within the block of 50cm alley and between blocks by one meter access route.

Samples of the poultry manure were subjected to chemical analysis and the results were presented in Table 2.

The experimental site was under a two year fallow after a two year continuous production of *Telfaria occidentalis* and *Solanum species*.

In the first year of the trial, 2004, the experimental site was cleared, ploughed and experimental plots established. In subsequent years, clearing was done plot by plot. After clearing, the debris including the residues of the various crops which were left after their harvestable portions had been harvested were ploughed into the soil within each plot.

Both cassava and the companion corps were planted on the same day. The cassava cuttings (20cm) were planted slanting at the spacing of 1m x 1m giving a population of 10,000 stands/ha. Planting spacing for melon was 50cm x 50cm giving a population of 40,000 stands/hectare while that of maize was 90cm x 90cm giving a population of 12,345.7 stands/ha. Each plot received 12kg of Poultry manure. Based on the randomization scheme, the manure was spread uniformly on the appropriate plots and then manually incorporated into the soil by tilling to a depth of 30cm using garden fork. Planting was done, 4 days after manure incorporation thereby allowing the manure to set properly.

The farm was weeded manually using hoe, only once at 4 weeks after planting. Samples of weeds were collected using 1m x 1m quadrant, dried and weighed for all treatments for the 5 years of the trials. The weeds were oven dried at 60^{0}c for 30 hours and then weighed. The

alleys of the experimental site were slashed as the weeds come.

Data collection on weed biomass was at every fourth week starting from 4 WAP till 44th WAP. Parameters assessed were root yield of cassava, seed yield of maize and seed yield of melon. Post harvest soil physico-chemical properties of the experimental site were carried out to determine the nutrient status of the soil after each harvest for the five years of the experiment.

Mixture productivity of the various crop mixtures were evaluated to determine whether there was yield advantage in the intercropping systems compared to the sole crops of the individual crops.

Statistical analysis of data collected were carried out using standard analysis of variance(*Anyeagbu 1995*), *Gomez and Gomez (1984)*. The significance of the treatments was determined using standard f-test. To determine the significance of the difference between the means of the treatments, least significant difference (LSD) was computed at the 5% probability level.

Results And Discussion

Soil

The result of the pre- planting soil analysis of the experimental site is shown in Table 3 while the Post harvest soil chemical analysis is shown in Table 10. In all control plots except that of melon plots, the soil after 5 years of continuous cropping became highly acidic less than pH 4.0. Thus continuous cropping on a particular piece of land without any form of conservation renders the soil useless. The soils in these control plots were also low in nutrient contents after the 5 years of cropping. Conversely, the post harvest soil chemical properties showed that application of poultry manure increased the pH status of the soil. Thus application of poultry manure at the rate of 10 t ha^{-1} improved and sustained soil pH for the 5 years of continuous cropping. The mechanism responsible for the neutralization of acidity by organic manure has been proposed by several workers (*Bessho and Bello 1992*, *Yan et al 1993*, *Pocknee and Summer 1997* ,that increase in soil pH obtained on addition of organic materials to the soil was as a result of ion exchange reactions in which the terminal OH^-s of AL or Fe^{++} hydroxyl oxides are replaced by organic anions which are decomposition products of the manure such as Malate, Citrate and Tartrate. *Bessho and Bello (1992*) suggested that the ability of organic manure to increase soil pH was due to the presence of basic cations contained in the organic manure. *Natsher and Schwetnnann (1991)* reported that such basic cations could only be released upon microbial decarboxylation.

The observed (Table 10) improvement in Nitrogen, available phosphorus, organic carbon etc of residual soil nutrient status at 5 years post harvest period in poultry treatments confirmed earlier work by *Sauerlandt and Tietjen (1970), Hiscard 1993*, *Janadasa et al 1997* ,that residual effect of manure, could last well into the third or fourth year of cropping. Thus obtaining a 4 to 5 years sustained soil fertility with application of 10 t ha^{-1} of poultry manure in this trial, confirmed that the report of *Godfery (1976*) of farm yard manure produced a sustained increased in Carbon content.

Component Crop Yields

Melon Application of poultry manure significantly (P>0 05) increased the seed yield of melon, (Table 4). Irrespective of cropping systems, the seed yield of melon in all control plots dropped after 3 years of cropping. The resultant trend was an indication that melon alone could not sustain the soil fertility for a long period of time. In all cropping systems, the seed yield of melon stands received poultry manure increased linearly up to 5th year of cropping. Perhaps the effectiveness of the residual effects of poultry manure was further enhanced by melon. In cassava/melon intercrop, melon stands that received poultry manure produced 38% more seeds than those in the control plots while in maize/melon system, stands of melon that received poultry manure produced 40% more seed than those in the control plots. In melon sole crop areas, stands given poultry manure gave 34% more yield than those in control plots. (Table 4). For the 5 years of trial, the highest seed yield a melon was recorded in cassava/melon intercrop. The reduction in the yield of melon in maize/melon inter crop may be attributed to stronger inter-

specific competition. *Anyaegbu (2008*) attributed the decrease in the root yield of cassava in cassava/maize mixture to competition for growth resources.

Cassava

Application of poultry manure significantly (P>0.05) increased the yield of cassava, (Table 5), irrespective of cropping systems. In control plots, the root yield of cassava decreased steadily with continuous cropping. This was due to depletion of soil nutrients without replacement. In the poultry manure treated cropping systems, cassava/maize and cassava sole crop respectively , the root yield of cassava dropped after the 4th year of cropping but in cassava/melon system, the root yield of cassava increased linearly up to 5th year of cropping (Table 5). In the above situation, intercropping with melon may have boosted the sustainability of the poultry manure in residual nutrient content of the soil. *Fagbamiye (1977) and Ikeorgu (1984)* had reported that melon *(planophile*) improved the yield of companion crops by conserving soil moisture and reducing high noon temperature, thereby making the environment more conducive for plant growth and development.

In maize/cassava mixture and cassava/melon mixture respectively, cassava stands given poultry manure produced 50% more "roots" than those in the control plots while in cassava sole cropping, cassava stands that received poultry manure gave 56% more yield than those in the control plots. Generally, in the 5 years of the trial, the highest root yield was recorded in the cassava/melon intercrop while the lowest was recorded from cassava/maize mixture. *Tran and Nguyen (2001*) in their trial on association of cassava with short duration crops, in South Vietnam reported that the yield of cassava in cassava/ maize intercrop was significantly reduced by competition for nutrients and water or by shading out by tall maize plants.

The seed yield of maize as affected by the 5 year residual effect of poultry manure application is shown on Table 6. Application of Poultry manure significantly (P>0.05) affected the seed yield of maize. In all control plots, the seed yield of maize was generally low and decreased steadily with the 5 year of continuous cropping. In the cassava/maize mixture and sole crop maize where poultry maize was applied, the seed yield of maize increased linearly up to the 3rd year beyond which it decreased steadily (Table 6).

Weed Biomass (G/M^2)

Weed density was significantly high in areas that received poultry manure (P>0.05) compared with the control plots (Table 7). In all cropping systems, weed density decreased steadily after the 3rd year of planting. This trend may be in line with the decreasing fertility status of the soil due to the continuous cropping. The lowest weed weight (68.8g/m) in control plots and(83g/m$^{2)}$ in manure treated areas was recorded in cassava/melon intercrops.

This reduced weed weight in melon/cassava mixture may be attributed to rapid vegetative development of melon with its canopy shading greater area of the plot thus reducing weed infestation. *Zuofa et al (1992)* found 16% and 40% reduction in weediness by intercropping with low growing smoother crops such as groundnut, cowpea or melon.. Thus the advantages of the live-mulching from melon include weed suppression, return of organic matter to the soil and conservation.

Mixture Production

The ratio for combination of cassava and the component crops in 5 year study gives a comparison of intercropping systems with sole cropping, (Table 8) The land equivalent ratio values for intercrops with all the respective combinations were greater than unity in all the seasons. While the yield advantage in this study ranged from 25% to 36% in 2004, 18% to 48% in 2005, 13% to 50% in 2006, 11% to 53% in 2007, it ranged from 11% to 56% in 2008. Averaged over the 5 growing seasons, the highest LER of 1.36 2004, 1.48 in 2005, 1.50 in 2006, 1.53 in 2007 and 1.56 in 2008 were obtained when cassava was intercropped with melon. Melon serving as a cover crop conserved soil moisture, reduced temperature and added organic matter to the soil through its leaves and these were advantageous to the mixture.

Thus the total LER of the mixtures being above 1.0 indicates that higher productivity per unit

area was achieved by intercropping. *Njoku et al (2007)* observed similar results in sweet potato/okra intercropping.

Gross Margin Analysis

In economic terms, cassava intercrops gave more Net returns and Benefit Cost Ratio over sole cropping (Table 9). *Willey (1979)* observed that practical significance of productivity in intercropping could only be fully assessed when related to the actual economic or monetary returns. Throughout the 5 years of trial, highest BCR (43) was obtained from cassava/melon intercropped that received 10 t ha^{-1} of poultry manure application.

Generally, except in cassava/melon intercrop treated with 10 t ha^{-1} of poultry manure, the BCRs in 2004 were higher than those in 2008. This gradual decrease may be associated with the gradual reduction in the residual effect of the manure with time. Moreover, the variable costs of each enterprise (treatment) was higher in 2008 than in 2004. This also may have contributed in the lower BCR experienced in the last year of the trial (2008) compared to those obtained in the first year of the trial (2004).

Table 1. Details of Treatment Combinations

Treatment Combinations	Manure Quantity
Cass + Maize + O (T_1)	Zero manure Application
Cass + Mel + O (T_2)	Zero manure Application
Cass + Maize + 10 t/ha (T_3)	10 t/ha manure applied
Cass + Mel + 10 t/ ha (T_4)	10 t/ha manure applied
Sole cass + O (T_5)	Zero manure application
Sole cass + 10 t/ha (T_6)	10 t/ha manure applied
Sole maize + O (T_7)	Zero manure
Sole Maize + 10 t/ha (T_8)	10 t/ha manure applied
Sole Melon + O (T_9)	Zero manure
Sole melon + 10 t/ha (T_{10})	10 t/ha manure applied

Table 2. Chemical composition of the poultry

Elements	Quantity
N (%)	1.95
P (%)	1.30
K (%)	0.82
Ca (%)	0.07

Na (%)	0.52
Mg(mg/kg)	25.05
Zn(mg/kg)	33.31
Cu(mg/kg)	30.61
Org. C (%)	27.15
Org. matter(%)	50.53
C:N ratio	19.8:1

Table 3. Pre-planting soil physico-chemical analysis of experimental site in 2004

Properties	Values
PH (H_20)	4.3
%Organic Carbon	1.20
% total Nitrogen	0.33
Exch. K+ (Cmol/Kg^{-1})	0.09
Avail P. (ppm)	14.00
CEC (Cmol/Kg^{-1})	4.91

Table 4. Seed yield of melon as affected by poultry manure under different cropping systems for 5 years of continuous cropping. Melon Seed Yield (t/ha)

	Cassava/melon		Maize+melon		Melon Sole crop		
Years	0	10 t/ha	0	10 t/ha	0	10 t/ha	LSD(P>0.05)
1	2.6	5.6	3.5	4.8	2.8	4.8	0.246
2	3.2	6.8	2.7	5.6	3.7	6.7	1.133
3	3.6	7.2	2.3	6.3	3.7	7.3	2.200
4	3.2	7.8	1.8	5.8	2.8	6.5	2.211
5	3.0	7.8	1.6	5.3	2.4	5.7	2.012
Mean	3.12	7.04	2.38	5.56	3.08	6.2	
LSD(P>0.05)	2.214		2.038		1.647		

Table 5. Yield of cassava as affected by Poultry manure under different cropping systems for 5 years of continuous cropping.

	Cassava/maize		Maize + Melon		Cassava Sole crop		LSD
Year	0	10 t/ha	0	10 t/ha	0	10t/ha	LSD(P>0.05)
1	10.6	20.1	10.7	28.6	11.6	20	3.611
2	7.8	23.6	14.2	38.3	10.4	24.6	4.302
3	7.2	25.4	14.2	41.5	8.3	33.5	4.544
4	6.8	25.8	14.2	42.8	6.2	35.6	5.017
5	6.3	23.2	12.6	44.2	4.9	30.6	8.333
Mean	7.7	23.6	13.2	39.1	8.3	28.9	
LSD(P>0.05)	4.817		7.325		4.590		

Table 6. Seed yield of maize as affected by poultry manure application under different cropping systems for 5 years of continuous cropping.

	Cassava/maize		Maize/melon		Cassava maize		LSD
Year	0	10 t/ha	0	10 t/ha	0	10t/ha	LSD(P>0.05)
1	2.0	3.2	2.6	3.6	2.8	3.4	0.167
2	1.5	3.8	2.2	3.8	2.1	4.2	1.001
3	1.2	4.1	2.2	4.2	1.6	4.5	1.235
4	0.8	3.5	2.0	4.5	1.4	4.0	1.014
5	0.6	3.4	2.0	3.8	0.8	3.6	1.240
Mean	1.2	3.6	2.2	4.0	1.7	3.9	
LSD(P>0.05)	0.314		0.623		0.481		

Table 7. Weed biomass (g/m2) as affected by poultry manure over a period of 5 years cropping systems.

	Cassava/maize		Maize/melon		Sole cassava		Sole melon		Sole maize	
Year	0	10	0	10	0	10	0	10	0	10
1	210	225	77	91	241	254	118	214	256	338
2	208	231	74	84	238	248	106	208	248	324

3	204	215	68	83	224	236	98.2	118	242	318
4	189	213	64	81	218	228	88.3	172	214	286
5	142	203	61	76	219	221	712	168	205	254
Mean	190.6	217.4	68.8	83	228	237.4	96.3	190	233	304
LSD(P>0.05)	12.017		11.214		14.282		5.213		21.630	24.619

Table 8. Mixture Productivity of various Component crops assessed by LER as influenced by poultry manure for 5 years of continuous cropping.

Crop combinations	Poultry rate	LER Years of Cropping					LSD(p>0.05)
–		1	2	3	4	5	
Cass + Maize	0	1.25	1.18	1.13	1.11	1.11	0.020
Cass + Mel +	0	1.31	1.24	1.27	1.20	1.18	0.021
Cass + Maize +	10	1.20	1.23	1.25	1.39	1.27	0.022
Cass + Melon +	10	1.36	1.48	1.50	1.53	1.56	0.23
Sole cassava	0	1.0	1.0	1.0	1.0	1.0	-
Sole cassava	10	1.0	1.0	1.0	1.0	1.0	-
Sole maize	0	1.0	1.0	1.0	1.0	1.0	-
Sole maize	10	1.0	1.0	1.0	1.0	1.0	-
Sole melon	0	1.0	1.0	1.0	1.0	1.0	-
Sole melon	10	1.0	1.0	1.0	1.0	1.0	-

Table 9. Gross margin analysis of the different systems/ha 2004 & 2008.

Cropping systems Poultry rates	Total Revenue 000(₦)		Variable Cost 000 (₦)		Gross Margin ,000 (₦)		Benefit Cost Ratio	
	2004	2008	2004	2008	2004	2008	2004	2008
Cass + Maize + 0	342	204	33	36	309	168	9.4	4.7
Cass + Mel + 0	236	216	25	27	211	189	8.4	7.0
Cass + Maize + 10 t/ha	1,075	482	44	46	1031	336	23.4	7.3

Cass + Melon + 10 t/ha	652	764	27	28	624	736	23.1	26.3
Sole cassava + 0	227	184	25	27	202	157	8.1	5.8
Sole cassava + 10	404	335	26	28	378	307	14.5	11
Sole maize + 0	84	63	20	21	63	42	3.1	1.6
Sole maize + 10 t/ha	186	143	22	25	164	116	7.5	4.6
Sole melon + 0	103	98	15	16	108	83	7.2	5.2
Sole melon + 10 t/ha	221	207	21	21	200	186	9.5	8.9

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Correlation and Paths Analysis between Stem Diameter and other Juvenile Growths Traits in Twelve Gum Arabic (*Acacia senegal* (L) Willd) Provenances

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Abstracts: Twelve provenance of *Acacia senegal* were obtained from their natural habitats in Sudan and Sahelian Ecological Zone of Yobe State. The seeds were sown in poly bags and laid out in a Randomized Complete Block Design replicated three times at the main nursery of Rubber Research Institute of Nigeria, Gum Arabic Sub-Station Gashua. Seedling traits were measured and subjected to correlation and paths analysis to ascertain their possible use in *Acacia senegal* future crop improvement programmes. There was positive and highly significant genotypic association between seedlings height, root spread and stem length with stem diameter. Root spread had the highest positive direct effects on stem diameter followed by number of primary branches and stem length. Therefore, since stem diameter is very important to gum production in *Acacia senegal*, the positive and significant association of these traits such as seedling height, stem length, and root spread hold promise in the improvement of gum yield. [Report and opinion 2009;1(6):87-90]. (ISSN 1553-9873).

Keywords: *Acacia senegal* (L) Willd, provenance, seedling traits, genotypic and phenotypic correlation, paths coefficients.

1. Introduction

Nigeria is one of the most naturally endowed nations in the world with 93,700 square kilometers estimated land mass for agriculture including gum arabic (*Acacia senegal* L.Willd).The crop is economically and ecologically important specie. It produces gum, which is highly sought for its multifunctional utilization in industries such as pharmaceutical, beverages, confectionery, textile among others (Bell *et al*,1994; Anderson and Weiping, 1992).The tree improves soil mineral content through symbiosis with rhizobium (Ojiekpon, *et al*,2007) and is among the local woody specie used in agroforestry systems and in desertification control through sand dune stabilization and as wind breaks (Cossalter, 1991).*Acacia senegal* demonstrates great intraspecific variability with respect to gum production(Motlagh *et al*., 2006). Appropriate tapping time is governed by tree age of 5-7years old with a stem diameter of about 5cm and a plant height of 1.2-1.5m depending on management practices (Jamal and Huntsinger, 1993.).Annual yield from young trees may range from 188-286kg (5-7years) from older trees 379-754kg (in about 8-15years) (Hine and Eckman, 1993).

The ultimate goal of *Acacia senegal* breeding is to improve the specie gum quantity and quality. This could be achieved through selection of superior genotypes which is always pursued. Stem diameter is an important trait considered for gum exploitation and is associated with a number of component characters, which in turn are interrelated. As more traits are included in the correlation studies, the inherent association become complex. For this reason, paths analysis becomes necessary. This is because it measures the direct and indirect influence of one variable upon another and permits the separation of relative contribution of different traits to the traits of measured interest.

Correlation and paths analysis, though frequently used in many agricultural crops, has only recently been used in tree crops (Khosla *et al.*, 1985; Siddiqui *et al.*, 1993; Srivastava and Chauhan, 1996; Gera *et al.*, 1999). Abraham *et al*, (2008) observed positive correlation between seedling height and root collar diameter (rp = 0.95 and rg = 1.00) in *Millettia ferruginea*. Similarly, Mahadevan *et al*, (1999) reported higher positive correlation in juvenile diameter with both height and diameter of mature trees in *Casuarina equisetifolia*. Rongling *et al*, (2004) observed dependant relationship that increased stem diameter due to assimilates supplies attributable to both canopy spread and foliage.

Due to long gestation period of trees, the analysis of juvenile growths traits is an important technique to establish the relative importance of different genotype as the determinant for improvement (Chaturvedi and Pandey, 2005). Also Burley and Wood (1978) observed that if good correlations exist between measured traits at different stages of the tree's development, prediction of growth at an advanced age may be possible. The study aims at selection of better plants with higher stem diameter based on correlation with other juvenile growths traits of *Acacia senegal*.

2. Materials and Methods

Acacia senegal germplasm were collected from 12 provenances in Yobe state in 2007, covering the two principal ecological zones of the

state with strong evidence of the species. Yusufari and Gashua for sahelian ecology while Damaturu and Gujba represented the sudan ecological zone. The selection criteria were based on the provenance trial recommendations (Burley and Wood, 1978).

The experiment was conducted in the nursery of Rubber Research Institute of Nigeria, Gum Arabic Sub- Station Gashua, located at latitute12^0 46'N, longitude 11^0 00' E and altitude 360m above sea level. Polythene bags measuring 7.5cm X 20cm were filled with a well decomposed potting mixture (2part topsoil: 2 part rivers and 1 part cowdung) and watered once to ease carriage and stacked in a Randomized Complete Block Design (RCBD) in three replications. Each treatment consisted of 30 polythene bags with 30cm X 50cm spacing between treatments and replication respectively. The stacked polypots were watered thoroughly for five days prior to sowing using watering can to soak and stabilize the soil. To enhance vigorous germination and development, pre-germination treatment was done by soaking seeds in tap water for 24hours at room temperature before sowing. 2–3 seeds were sown by hand at a depth of 1cm and were thinned down to one plant per polypot. The nursery was kept weed free throughout the experiment. Data was collected from 5 seedlings taking at random. These include: Emergence count, seedling height (cm), stem length (stem height at first branching) (cm), no of primary branches, canopy spread (cm), root length (cm), root spread (cm), and stem diameter (cm). The correlation and paths analysis were performed as suggested by Singh and Chaudhary (1985).

The correlation coefficients between different pairs of traits were determined at genotypic and phenotypic levels. The path coefficient analysis which is simply a standardized partial regression coefficient which divides the correlation coefficients into direct and indirect contributions of independent variables on the dependent variable (stem diameter) was determined.

3. Results and Discussion

In general, the genotypic correlation coefficient values were higher than corresponding phenotypic values (*Table1*): This could attribute to environmental influence inherent in the phenotypic correlation (Chaturvedi and Pandey, 2005).The genotypic correlation is an estimated value whereas phenotypic correlation is an estimated value from the genotype and environmental interactions. This explains why genotypic correlation is a more reliable estimate for examining the degree of relationship between pairs of character (Chaturvedi and Pandey, 2005).

Emergence count had negative and highly significant genotypic correlations with seedling height, stem length, stem diameter and root length. With number of primary branches, the correlation was positive and highly significant. Seedling height had highly significant genotypic correlation with stem diameter (r=0.968) and canopy spread(r=0.746).The phenotypic level also showed positive and highly significant correlation with stem diameter(r=0.729) and significant correlation with canopy spread(r=0.673).Stem length revealed positive significant genotypic correlation with stem diameter(r=0.660)and canopy spread(r=0.578).This type of relationship implies that stem growths may be as a result of assimilates supplied as influenced by canopy spread and foliage(Rongling *et al*, (2004).Root spread exhibited highly significant genotypic correlation with stem diameter(r=0.975), canopy spread (r=0.903) and root length(r=0.780), whereas only canopy spread showed positive association at the phenotypic level. Three traits, seedling height, canopy spread and root spread present evidence of positive and highly significant genetic correlation with stem diameter whilst two traits, seedling height and canopy spread were positively and highly significantly correlated with stem diameter at phenotypic level. However stem diameter had positive genotypic correlation with all the traits except emergence count, number of primary branches and root length. Such result has also been reported by Chaturvedi and Pandey (2005) in *Bombax ceiba;* Siddiqui *et al* (1993) in *Terminalia specie.* In *Acacia senegal*, one important traits considered for gum exploitation is stem diameter and traits positively and significantly associated with stem diameter such as seedling height, stem length, canopy spread and root spread are therefore of interest to the breeder, since selection of one or more of these traits is likely to improve the gum yield, if such correlation is maintained up to maturity stage of the tree.

This type of result suggests that selection of root spread for stem diameter improvement in *Acacia senegal* may be worthwhile. Stem length at first branching had a direct positive effects on stem diameter and interrelationship(r=1.942 and r=0.660) respectively. Number of primary branches(r=3.505) had direct positive effects but recorded negative indirect effects with most of the other traits. Therefore, direct selection through this trait might be helpful in *Acacia senegal* improvement. The characters such as emergence counts, seedling height and canopy spread, although recorded a significant positive correlation, their direct effects were negative and this is due a higher negative values of the indirect effects of the other traits. In the same vain root spread had a highly significant correlation(r= 0.976) with stem diameter, but its direct effects was about six times the correlation value(r= 6.724) and this was due to the positive indirect effects of canopy spread and stem diameter. The correlation values of emergence count and its direct effects are both negative(r= -0.229 and r= -1.164, respectively).This type of

result suggested that for improvement in stem diameter, selection for the emergence count should be increased. Such results are similar to the findings of Jindal, *et al*. (1987) in *Acacia senegal*.

Generally, root spread was identified as an important trait for *Acacia senegal* improvement in this study followed by stem length at first branching. This is due to the fact that apart from their highly significant genotypic and phenotypic correlations, they also had positive direct effects on stem diameter.

Table1: Genotypic (upper diagonal) and phenotypic (lower diagonal) correlation coefficients between eight quantitative growth characters in 3month old *Acacia senegal* seedlings

Characters	Emergence count	Seedling height (cm)	Stem length (cm)	No. of primary branches	Canopy spread (cm)	Root length (cm)	Root spread (cm).	Stem diameter (cm)
Emergence count	1	-0.905	-0.105	0.762**	0.520	-0.922	-0.576	-0.886
Seedling height (cm)	-0.324	1	0.298	0.009	0.747**	0.536	0.551	0.968**
Stem length (cm)	-0.515	0.411	1	-0.652	0.578*	0.405	0.447	0.660*
No. of primary branches	0.426	0.202	0.364	1	-0.254	-0.206	-0.455	-0.229
Canopy spread (cm)	0.427	0.673*	0.344	-0.032	1	0.504	0.904**	0.378
Root length (cm)	-0.179	0.538	0.254	-0.072	0.464	1	0.780**	0.541
Root spread (cm)	-0.475	0.414	0.306	-0.283	0.614*	0.377	1	0.976**
Stem diameter (cm)	-0.385	0.729**	0.338	0.020	0.816**	0.440	0.531	1

*, ** significant at P= 0.05 and 0.01, respectively.

Paths analysis revealed that at the genotypic level root spread had the highest positive direct effects(r=6.724) and greatest contribution with stem diameter (r=0.976) *(table2)*.

Table2**:** Direct (highlighted) and indirect effects of seedling growths traits on stem diameter

Variables	Emergence count	Seedling height (cm)	Stem length (cm)	No. of primary branches	Canopy spread (cm)	Root length (cm)	Root spread (cm)	Genotypic correlation
Emergence count	**-1.164**	0.323	-1.975	2.672	-1.102	4.230	-3.057	-0.886
Seedling height (cm)	1.053	**-0.357**	0.578	0.033	-1.583	-2.459	3.703	0.968**
Stem length (cm)	1.184	-0.106	**1.942**	-2.287	-1.226	-1.856	3.009	0.660*
No. of primary branches	-0.888	-0.003	-1.267	**3.505**	0.538	0.944	-3.057	-0.229
Canopy spread (cm)	-0.605	-0.267	1.122	-0.889	**-2.121**	-2.311	6.077	0.378
Root length (cm)	1.074	-0.192	0.786	-0.721	-1.069	**-4.586**	5.249	0.541
Root spread (cm)	0.670	-0.197	0.869	-1.594	-1.917	-3.580	**6.724**	0.976**

Residual effects = -1.45202

*, ** significant at P= 0.05 and 0.01, respectively.

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Dietary Methionine Requirement Of *C. Gariepinus* Fingerlings And Its Effect On The Growth And Body Composition

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Abstract: The experiment was conducted to determine the methionine requirement for *Clarias gariepinus* and its effect on growth and body composition. Hatchery bred fingerlings of *C. gariepinus* (2.97±.036g) were stocked in eighteen 54L glass aquaria. Six diets (40% crude protein) consisting of a basal diet containing 1.81g methionine/100g protein from the ingredients soyabean and guinea corn was formulated, a reference diet (3.12g methionine/ 100g protein) and others having graded levels of crystalline methionine (2.87, 2.97, 3.00 and 3.07g/100g protein). Each diet was fed to three aquaria twice daily (8.00 – 18.00 hrs) for 56 days. The mean weight gain, specific growth rate, food conversion efficiency and protein efficiency ratio were significantly influenced by the level of methionine ($P<0.05$). Second order polynomial regression analysis showed that the requirement of *C. gariepinus* for methionine is 2.97g/100g protein. Carcass protein showed an increase above that of the initial fish stocked before feeding commenced. The percentage lipid also increased except for the fish fed 3.07 g methionine/ 100g protein. [Report and Opinion 2009;1(6):91-95].(ISSN:1553-9873)

Keyword: Clarias fingerlings, methionine, body composition, requirement

1. Introduction

In feed formulation the present trend and most beneficial is to consider least-cost, with low fish meal and high level of plant proteins. All plant proteins contain anti-nutritional compounds (NRC 1993) which limit bioavailability of some amino acids (Cai and Burtle 1996). Methionine and lysine are the limiting essential amino acids in plant based fish diets. Methinine also serves as a precursor to carnitine (Tacon 1990). It is also required for building muscles and detoxifying the liver (Health Vitamin Guide.com). Its availability in the appropriate quality is important.. The methionine requirements of several species have also been reported (Table 1).

According to Lim and Dominy (1989) a protein with an essential amino acid composition which closely matches the essential amino acid requirements of the fish is described as being of high nutritive value. A protein that is deficient in one or more essential amino acids is of low biological value. Previous analytical work on the whole body protein of *C. gariepinus* shows that methionine makes up 2.77g/100g protein of the fish (Fagbenro *et al*., 2001). This study was conducted to determine the methionine requirement and its effect on the body composition of *Clarias gariepinus* fingerlings

Table 1. Quantitative methionine requirement of various fish species

Fish Species	Methionine requirement	Reference
Channel catfish	9.4g/kg	Cai and Burtle (1996)
Catla catla	3.55g/100g protein	Ravi and Devaraj (1991)
Seriola quinqueradiata	2.56g/100g protein	Ruchinat et al., (1997)
Pseudosciaena crocea	3.34g/100g protein	Mai et al., (2005)
Cirrhinus mrigala	3g/100g protein	Ahmed et al., (2006)
Epinephelus coioides	2.73g/100 protein	Luo et al., (2005)
Rachycentron canadum	2.64g/100g protein	Zhou et al.,(2005)
Dicentrachus labrax	2.0g/100g protein	Thebault et al., (1985)
Oncorhynchus mykiss	2.2g/100g protein	Walton et al., (1982)
Channel catfish	2.3g/100g protein	Nose (1989)
Oreochromis niloticus	2.7g/100g protein	Santiago and Lovell (1988)
Anguilla japonicus	2.9g/100g protein	Harding et al., (1977)
Cyprinus carpio	3.1g/100g protein	Harding et al., (1977)
Clarias gariepinus	3.2g/100g protein	Fagbenro et al., (1998)
Sparus aurata	4.0g/100g protein	Walton et al., (1982)
Seriola quinqueradiata	3.1-3.4g/100g protein	Twibell et al., (1999)

2.0 Experimental design, feeding and Sampling

Clarias gariepinus fingerlings (initial mean weight 2.97 ± 0.36g) were stocked 6 fish per glass aquaria (54L) for six treatments. Each treatment was replicated thrice in a 2 x 3 x 6 factorial experimental design. The fish were fed five diets which were having ingredients that are methionine deficient (Guinea corn 0.85 + soyabean 0.96 = 1.81g/100g protein). The methionine content of *C. gariepinus* is 2.77g/100g protein. A basal diet containing 1.81g methionine/100g protein was formulated. The proximate composition of the ingredients was analyzed as in Table 2. The experimental diets were supplied with 2.87g, 2.97g, 3.00g and 3.07g methionine/100g protein respectively (Table 3). A reference diet was formulated with fish meal and groundnut cake in addition to the ingredients in the basal diets. They were pelleted and sun dried for three days. The diets were fed to three replicates of fish stocked in 54L glass aquaria twice daily for 56 days.

Sampling was carried out biweekly by bulk weighing the fish. On sampling days complete replacement of water was carried out, while on other days remnant of feed and faecal wastes were siphoned out. Partial replacement of water was done on those days.

2.1 Chemical and Statistical analysis

At the beginning of the experiment five pieces of fish was analyzed for their chemical composition. At the end of the experiment five pieces of fish from each treatment was also analyzed (AOAC 2000). Proximate analysis of feed was also carried out according to AOAC (2000). The amino acid composition of the diets was analyzed using Technicon Sequential Multisample Amino Acid Analyzer (TSM – 1 model DNA 0209) (Table 5).

Table 2. Proximate Composition Of Ingredients

Ingredients	% Moisture	% Protein	% Lipid	% Ash	% Crude Fibre	% NFE
Fishmeal	4.81	63.44	21.95	9.00	0.80	Neg.
Soyabean	1.73	43.49	34.60	6.26	6.80	7.12
Guinea corn	6.93	11.17	17.10	2.18	1.60	61.02
Groundnut cake	4.85	41.02	41.30	5.65	0.70	6.48

Table 3. Percentage composition of diets

	Soyabean	Fish meal	Groundnut cake	Guinea corn	Premix	Starch	Oil	Methionine
DIET I	758.30	-	-	91.70	30.00	20.00	100.00	-
DIET II	757.4	-	-	91.60	30.00	20.00	100.00	10.00
DIET III	755.6	-	-	91.40	30.00	20.00	100.00	30.00
DIET IV	753.8	-	-	91.20	30.00	20.00	100.00	50.00
DIET V	752.1	-	-	90.9	30.00	20.00	100.00	70.00
DIET VI	234.2	117.1	351.4	147.30	30.00	20.00	100.00	-

Aqua Biomix Fish Premix

Vitamin A	i.u	20,000,000	Vitamin B2	mg	30,000	Folic acid	mg	4,000
Vitamin D3	i. u	2,000,000	Niacin	mg	150,000	Biotin	mg	800
VitaminE	mg	200,000	Pantothenic acid	mg	50,000	Choline	mg	600,000
Vitamin C	mg	500,000				chloride		
Vitamin K3	mg	8,000	Vitamin B6	mg	12,000	Cobalt	mg	2,000
Vitamin B1	mg	20,000	Vitamin B12	mg	50	Copper	mg	4,000
Iodine	mg	5,000	Selenium	mg	200	Antioxidant	mg	100,000
Inositol	mg	200,000	Zinc	mg	40,000	Lysine	mg	100,000
Iron	mg	40,000	Manganese	mg	30,000	Methionine	mg	100,000

Table 4. Proximate Composition Of Diets

	% Moisture	% Crude Protein	% Crude Lipid	% Ash	% Crude Fibre	% NFE
DIET I	4.02	40.01	24.18	7.45	1.04	23.3
DIET II	3.43	40.00	23.98	8.62	6.24	17.73
DIET III	3.38	40.83	24.10	6.82	2.79	22.08
DIET IV	3.56	40.12	23.94	8.26	4.08	20.04
DIET V	4.14	40.80	24.24	7.63	3.93	19.98
DIET VI	3.72	40.67	24.19	8.43	2.47	20.52

Table 5. Essential Amino acid composition of diets (g/100g protein)

Amino acid	DIET I	DIET II	DIET III	DIET IV	DIET V	DIET VI
Lysine	3.92	5.21	6.01	5.21	6.01	5.69
Histidine	3.32	2.51	2.38	2.13	2.44	3.01
Arginine	7.16	6.98	6.63	6.47	6.80	6.98
Threonine	2.89	2.72	2.55	2.68	3.21	3.00
Methionine	1.81	2.87	2.97	3.00	3.07	3.12
Isoleusine	4.07	4.20	3.95	3.85	4.33	4.20
Leusine	6.09	6.09	5.93	5.71	6.37	6.42
Tyrosine	3.22	3.38	3.22	3.06	3.38	3.54
Phenylalanine	4.06	4.22	4.39	4.05	4.56	4.73
Valine	3.95	4.01	4.30	3.89	4.01	4.18

Statistical analysis was carried out using computer package SPSS version 10, One – way Analysis of Variance (ANOVA) was utilized to test for significance of growth parameters. Regression graphs were drawn to show the relationship between level of methionine in diet and growth, food conversion efficiency and protein efficiency ratio.

3. Results Analysis

The quadratic regression graph of all growth parameters with level of methionine showed a maxima at 2.97g methionine/100 protein. The mean final weight, specific growth rate, protein efficiency ratio and food conversion efficiency varied significantly ($P<0.05$) (Table 6). Percentage growth of fish ranged from 96% to 155% for fish fed the varying levels of methionine. The growth of the fish fed the basal diet was not significantly different from the fish fed additional methionine ($P<0.05$).

The moisture, crude protein and fat content of all experimental fish were higher than initial. The ash and fibre content of the fish stocked were higher than that of the experimental fish. The crude protein content of all fish fed methionine was higher than the those fed the basal and reference diets. (Table 7)

Table 6. Growth of *C. gariepinus* fingerlings fed varying levels of methionine in 56 days

	Mean Initial Weight (g)	Mean Final Weight (g)	Mean Weight Gain (g)	Specific Growth Rate (%)	Protein Efficiency Ratio	Food Conversion Efficiency
DIET I	2.97±0.36	6.55±0.3 ^{a}	3.58±0.3^{a}	1.41±0.09^{ab}	1.02±0.05^{a}	0.033±0.004^{a}
DIET II	2.97±0.36	5.84±0.29 ^{a}	2.87±0.29^{a}	1.21±0.09^{a}	0.91±0.05^{a}	0.041±0.003^{bc}
DIET III	2.97±0.36	7.59±1.0 ^{a}	4.62±1.0^{a}	1.66±0.24^{b}	1.19±0.16^{a}	0.047±0.01^{cd}
DIET IV	2.97±0.36	6.02±1.28 ^{a}	3.05±1.28^{a}	1.35±0.31^{ab}	0.94±0.2^{a}	0.03±0.004^{a}
DIET V	2.97±0.36	6.46±1.07 ^{a}	3.99±0.81^{a}	1.37±0.3^{ab}	1.01±0.17^{a}	0.035±0.001^{ab}
DIET VI	2.97±0.36	12.04±1.34 ^{b}	9.07±1.34 ^{b}	2.5±0.19^{c}	1.88±0.21^{b}	0.053±0.01^{d}

Figures with the same superscript in a column are not significantly different ($P>0.05$)

Table 7. Carcass composition of *Clarias gariepinus* fingerlings fed varying levels of methionine in 56 days

	MOISTURE	% CRUDE PROTEIN	% CRUDE FAT	% ASH	% CRUDE FIBRE	NFE
DIET I	73.00	19.38	4.30	2.00	0.5	0.82
DIET I	74.05	20.29	3.05	1.85	0.6	0.16
DIET I	72.00	22.59	4.40	1.00	0.2	0.31
DIET I	72.60	21.67	4.00	1.65	0.6	Neg.
DIET I	73.30	21.11	3.30	2.15	0.7	Neg.
DIET I	72.30	19.88	4.05	1.10	0.54	Neg.
INITIAL	71.40	18.90	2.13	3.95	2.45	0.17

4. Conclusion

In using the second order quadratic regression equation derived from the graphs of growth of *C. gariepinus* and level of methionine the point at which dy/dx was equal to zero was 2.97g methionine /100g protein. This result corroborates the earlier experiment carried out with *C. gariepinus* fry (Ovie and Eze, in press). In both fry and fingerling experiments the regression graphs had maxima at 2.97g methionine/100 protein. This is similar to the observation for channel catfish (Wilson and Poe, 1985); Ahmed *et al*., (2006) for *Cirrhinus mrigala*, Zhi *et al*., (2005) for *Epinephelus coioides*; Ruchinat *et al*., (1997) and Twibell *et al*. (2000), for *Seriola quinqueradiata*; Harding *et al*., (1977) for *Anguilla japonicus* and *Cyprinus carpio*; Santaigoand Lovell (1988) for *O. niloticus*, Borlongan and Coloso (1993) for *Chanos chanos*; Jackson and Capper (1982) for *S. mossambicus*; Keembiyehetty and Gatlin, (1993) for hybrid striped bass; Moon and Gatlin, (1991) for *Sciaenops ocellatus*; Nose, (1979) for Carp and Rodehutscord *et al*., (1995) for *O. mykiss*. However, this does not compare well with the observation for red sea bream *Pagrus major*, whose amino acid requirement changed with growth and or age (Takagi *et al*., 2001). This study shows a slightly lower methionine level than that observed for the same species by Fagbenro *et al*. (1998b). The slight variation could be due to several factors such as fish size, culture protocols, model used, and basal diets composition (Bureau and Encarnacao, 2006). The fact that there was no significant difference in the growth of fish fed varying levels of methionine and that fed the basal diet shows that *C. gariepinus* grows appreciably with feed of low biological value. This result is similar to an earlier study on *C. gariepinus* fry (Ovie and Eze, In press). When the growth of fish of all diets containing methionine is compared with the fish fed the reference diet the latter is superior, an indication of the bioavailability of the essential amino acid than that of the experimental diets (Miles and Chapman, 2007). This result is similar to the observation made for *Ictalurus punctatus* that supplementation of methionine in low protein but amino acid replete diets were not beneficial (Li and Robinson, 1998). The percentage weight gain in this study exceeds that reported by Twibell *et al*., (2000) for *Seriola quinqueradiata* but lower than that reported by Fagbenro *et al*., (1998) for *C. gariepinus.*

The carcass composition of the fish fed varying levels of methionine varied significantly ($P<0.05$). This agrees with Ahmed *et al*., (2003) for *Cirrhinus mrigala*. Fish fed methionine above or below the requirement level did not show any nutritional defects. The increase in the methionine content in the diets is not reflected in the protein content of the body, however, all fish fed crystalline amino acid, i.e. methionine showed higher protein content than that fed basal and the reference diets

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Salinity and Arsenic Threat in the irrigated fields of Mekelle Plateau of the northern Highlands of Ethiopia

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Abstract: Due to its semi arid climate, the Tigray Plateau suffers from chronic water shortage. In a country where agriculture represents the major form of income, ensuring adequate water becomes especially important. This concern was addressed historically by the construction of above 60 community dams throughout Tigray. This study was conducted to evaluate the impact of the recently constructed community dams on soil salinisation and arsenic accumulation in Mekelle Plateau. Hence, a total of twenty seven soil samples and nine water samples were collected and sent to the Analytical Services Laboratory of the International Livestock Research Institute (ILRI-Ethiopia) for examining the magnitude and severity of soil salinity and arsenic concentration. Accordingly, all the water samples in these dams contain salts, which the TDS varied as little as 147 µg ml^{-1} in *Adigudom dam* to 236.8 µg ml^{-1} in *Gerebsegen dam*. Based on the ratio of soluble sodium percentage to salt concentrations, *May Gasa* and *Gum Selasa* dams were the most salted dams with the amounts of 34.6 and 30%, respectively. Fifty nine percent of the studied soils were saline with the salt level greater than 2 dSm^{-1} at which the growth of major cereal crops can be impaired and the remaining 49 % were greater than 1.25 dSm^{-1}, which are potentially hazardous. The arsenic concentrations in all the soil samples were extremely high and were varied from 260-440, 260-300 and 260-460 ppm in soils of Korer, Gum Selasa and Kelamino irrigated fields, respectively. [Report and Opinion 2009;1(6):96-102].(ISSN:1553-9873).

Keywords: Arsenic, irrigated fields, Mekelle Plateau, north Ethiopia, salinity

1. Introduction

Successful agricultural development will result in a significant reduction of poverty and an improvement in food security. However, in Ethiopia, despite numerous macroeconomic, political, and sectoral reforms, poverty, environmental degradation and food insecurity appear to be on the rise. There is a pressing need for Ethiopia to increase agricultural productivity while pursuing sustainable management of their natural resource base on which food production depends. Such development efforts require significant public investments in agricultural and environmental resources development. Agriculture is the mainstay of Ethiopia's economy, providing the livelihood base for nearly 85% of the population, contributing over 50% of gross domestic product, and accounting for about 90% of foreign exchange earnings. Many would concur that the overall performance of Ethiopia's economy for the foreseeable future to a great extent depends on the developments in this sector. The lack of sustained economic growth and emergent food crises in the country now and in the past are the results of weak transformation of the agricultural sector. These conditions are buttressed by high population growth, environmental degradation, and poor market and institutional arrangements.

Ethiopian agriculture is largely small-scale, subsistence-oriented, and crucially dependent on rainfall. The highlands of Ethiopia, which house most of the country's agricultural potential, suffer from massive land degradation due to soil erosion caused by heavy runoff and deforestation, and the low productivity of peasant agriculture Grepperud (1996). The increasing loss of soil and other natural resources has resulted in steady declines in land and labour productivity (FAO, 1986; Hurni, 1993; Shiferaw and Holden, 1999).

Dependence on rainfed agriculture coupled with the erratic nature of rainfall is one of the main causes of widespread food insecurity in the country. Droughts occur every 3-5 years in northern Ethiopia and every 8-10 years for the whole country, with severe consequences for food production (Haile, 1988). With the lack of well-functioning social networks to provide safeguards at the local, regional or national levels, it is prohibitively difficult to survive even a single year of failed harvest. Hence a sustainable increase in food production to achieve self-sufficiency depends, at least in part, on how Ethiopia addresses its dependence on rainfall. To this effect, the construction of dams and development of irrigation schemes will provide many poor Ethiopian farmers with greater food security, an improved diet and

increased income. Accordingly, in the Tigray region of northern Ethiopia, an extensive community-led microdam-based irrigation scheme was in progress, managed by the Tigray Sustainable Agricultural and Environmental Rehabilitation Commission (SAERT 1994; WIC 2002). To date about 60 have been constructed ranging in size from 50 000 to 4000 000 m^3 (unpublished data), and most are situated near human settlements (Fig 1). Regular monitoring and critical analyses under various agro-ecological zones of the ecological impact of these dams is imperative and worthwhile for timely intervention and sustainable use of them. Apparently, irrigation adds salts to soil. Soil salinization in its early stages of development reduces soil productivity, but in advanced stages kills all vegetation and consequently transforms fertile and productive land to barren land, leading to loss of habitat and reduction of biodiversity. Moreover, salinization can damage the economy of salt affected countries. In Ethiopia, the Amibara Melka Sedi area, which covers about 14 200 ha of net irrigable land in the Awash River Basin, encounters problems of salinization and rising watertables to varying degrees. The estimated cost of the development program to introduce subsurface drainage and thereby reduce salinity and rising watertable hazards is about US$ 52.2 million (Office of the National Committee for Central Planning, 1988). The social cost of salinization is not easy to quantify. Salinisation causes occupational and geographic shifting of the farm population and reduction in aggregate national income and expenditure. These events have social and economic repercussions on the country as a whole. The impacts are most apparent in rural hamlets and small towns because the opportunities for adjustment of the local economic base are more limited (Peck *et al*., 1983). Hence, this paper is motivated to provide information on salinity and arsenic risk in the environs of nine selected dams in the Mekelle plateau.

a) b)

Figure. 1. a) A community dam constructed in one of the study areas b) white salt patches at the outlet of irrigated fields in Adigudom

2. Materials and Methods

2.1 The study area

The Mekele Plateau is the eastern-central portion of the northern uplands of Ethiopia, which is known as the Tigrean Plateau. At its centre is the city of Mekelle, the capital of the northern Ethiopian region of Tigray. The Mekelle Plateau is an upland plateau with elevations ranging between 2000 and 2800 m.a.s.l. The terrain is composed of an undulating and rolling plateau, steeply dissected hills and pediments, and flood plains. The drainage pattern is characterized by the scarcity of deeply incised river valleys. The underlying geology is dominated by Jurassic Agula shale and Hintalo limestone and Mekelle dolerite sills in the Agula shale. The Plateau lies in the semi-arid zone with an average annual rainfall of 550 mm Agriculture in the plateau is based on ox-plough cultivation of predominantly cereal crops. This technology has prevailed without modification for thousands of years, harvesting the same land over and over again. The level of subsistence, except for periods of good rains, has declined radically during the past decades, with almost everything produced being consumed at the farm household level. The cropping pattern is dominated by barley, wheat and teff (*Eragrotis teff*). Drought is a recurrent factor in the farming system and responses to it include a shift to low-yielding drought resistant varieties of traditional crops.

Recently, in this particular area, nine microdams were constructed to mitigate drought and develop small scale irrigation viz., *Mai Gassa dam*; *Adi Kefaniz dam*; *Feliglig dam*; *Duranbesa dam*; *Girat Shito dam*; *Mai Haidi dam*; *Gereb segen*; *Gum Selassa* and *Mai Delle* dams were selected for water quality studies whereas three dams namely, *Gumselasa*; *Kelamino* and *Qorer* dams were randomly selected to assess the distribution of arsenic. The dams and their location characteristics are illustrated in Fig 2.

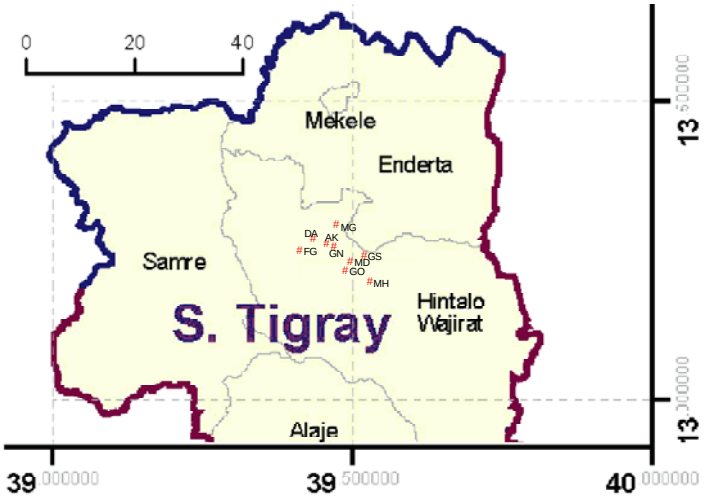

Figure. 2. Dams and their location in Mekelle Plateau where DA (Dur ambesa), MG (Mai Gasa), AK (*Adi Kefaniz*), FG (*Feliglig*), GN (*Gereb segen*), GS (*Gum Selassa*), MD (*Mai Delle*), GO (*Girat Shito*) and MH (*Mai Haidi*)

2.2 Methods

Nine profile pits were opened in the representative sites where the current irrigation practices are carried out (Fig 1). All the nine pits were morphologically described and twenty seven soil samples were collected for Lab analysis. Furthermore, nine water samples were collected from these dams. Both the water and soil samples were sent to the Analytical Services Laboratory of the International Livestock Research Institute (ILRI-Ethiopia) for examining the exchangeable Na, Mg and Ca. Bicarbonate, carbonate, sulphate and chloride were analyzed in the Soils Research Laboratory of Mekelle University. Finally, from a randomly selected three dams environs another twenty seven surface soil samples were collected to analyse the arsenic concentration. The soil samples were sent to the Ezana Commercial Mining Laboratory, Mekelle for arsenic determination using atomic absorption spectrophotometer (AAS). The electrical conductivity (EC) ($mScm^{-1}$) of the soils was determined using the equation: EC= (∑ cations, cmol kg^{-1} soils)/10 (U.S. Salinity Laboratory Staff 1954) and the sodium adsorption ratio (SAR) was calculated from the equation (Singer and Munns, 1987).

$$SAR = \frac{Na^{+}}{\sqrt{\frac{Ca^{+2} + Mg^{+2}}{2}}} \quad \text{[Eq.1]}$$

And the exchangeable sodium percentage of the soil (ESP) was calculated considering the percentage of exchangeable sodium ions to the total exchangeable cations of all types in the soil sample (Donahue *et al.*, 1983). Water quality in the dams was rated according to [Eq.2] as described in (LLoyd, *et al*., 1964). All waters that would cause equilibrium exchangeable sodium percentages of 15 or greater were assumed to be unsatisfactory for general use. Descriptive statistics was used to analyse the data.

$$(Na * 100) / (Ca + Mg + Na) \quad \text{[Eq.2]}$$

3. Results and Discussion

3.1 Water resource salinization

The quality of irrigation waters depends principally up on the total amount of salt present and the proportion of sodium to other cations (Wilcox, 1958). The most satisfactory method for rating the salt content of irrigation

waters involves measuring electrical conductivity. Hence, the EC of the water resource in the nine dams were varying from 230 in *Adigudom dam* to 350 µS cm^{-1} in *May Gasa dam* (Table 1). All the EC values are rated as moderate salinity hazard. All waters in these dams contain salts, which the TDS varied as little as 147 µg ml^{-1} in *Adigudom dam* to 236.8 µg ml^{-1} in *Gerebsegen dam*. Based on the ratio of soluble sodium percentage to salt concentrations, water resources from dams *May Gasa*, *Dur Anbesa*, *Gerebsegen*, *Girashito* and *Gum Selasa* were found unsatisfactory for general use as their values were greater than 15. The worst of all were waters from *May Gasa* and *Gum Selasa* dams since the values were 34.6 and 30%, respectively.

Table 1. Water resource salinization in the nine micro dams of Mekelle Plateau

Micro Dams	Na	K	Ca	Mg	EC	TDS	$\frac{(Na * 100)}{(Ca + Mg + Na)}$
	$mmol_c l^{-1}$				µS cm^{-1}	Mg L^{-1}	
May Gassa	1.22	0.02	1.65	0.66	355	227.2	34.6
Adi Karafiz	0.21	0.02	1.5	0.6	233	149.1	9.1
Filiglig	0.22	0.02	1.45	0.56	225	144.0	9.9
Dur Anbesa	0.36	0.02	1.44	0.53	235	150.4	15.4
Gerebsegen	0.71	0.04	2.35	0.59	369	236.2	19.4
Girashito	0.71	0.02	2.04	0.72	349	223.4	20.5
Mai Haidi	0.24	0.09	1.87	0.47	267	170.9	9.3
Gum Selasa	0.69	0.02	1.17	0.44	232	148.5	30.1
Maidelle	0.57	0.02	1.81	0.59	299	191.4	19.2
Mean±SD	0.55±0.33	0.03±0.02	1.69±0.36	0.57±0.08	284±59.3	182.3±38	18.6±9.05
Median	0.57	0.02	1.65	0.59	267	170.9	19.2
Min	0.21	0.02	1.17	0.44	225	144	9.1
Max	1.22	0.09	2.35	0.72	369	236.2	34.6

3.2 Soil resource salinization

A high salt level interferes with the germination of new seeds. Salinity acts like drought on plants, preventing roots from performing their osmotic activity where water and nutrients move from an area of low concentration into an area of high concentration. The actual damage done by salt to plants or soils depends on the concentration in the soil solution rather than on the quality in irrigation water (LLoyd, *et al*., 1964). Thus, use of same water source might lead to a severe salt problem in one soil where drainage is restricted and great concentration of salts occurs. As is shown in Table 2, 59 % of the investigated soils were found saline as they have the EC (dSm^{-1}) greater 2 and SAR and ESP values less than 13 and 15, respectively. Apparently, this range will impair the growth of sensitive crops. The highest EC (3.54 dSm^{-1}) was found in the Calcisols of Girat Shito at the depth of 100-150 cm and the lowest EC was 1.22 dSm^{-1} at the depth of 100-150 cm in the Luvisols of Mai Haidi. The highest surface soil EC found on the Vertisols of May Gassa, which was 2.56 dSm^{-1} followed by the Vertisols of Gerebsegen that was 2.52 dSm^{-1}. Concurrently, the sodium hazard is dangerously emerging in the Vertisols of Girat Shito where the highest ESP (12.28%) was observed at the upper horizon followed by Vertisols in May Gassa, which was ranged from 9.64 in the surface to 10.35% at the depth of 100 cm. Detrimental effects of high exchangeable sodium on plant growth occur because of poor soil physical condition. Some plants, however, begin to show some injury at levels as low as 5 percent exchangeable sodium. This level of sodium apparently has started to cause soil structural disturbance (**Lamond, R.E. and David A. W., 1992**). Notably, the anions are equally important in affecting the growth potential of the plants. As is shown in Table 3, though all the anions are in safe range the anionic composition of the soils of the study areas are in the order of importance $HCO_3^- + CO_3^{2-} > SO_4^{2-} > Cl^-$.

Table 2. Cationic composition, salinity and sodicity parameters of the soils of the study areas

Soil Depth cm	Na^+ Cmolc kg^{-1}	Ca^{2+}	Mg^{2+}	SAR	ESP %	EC dS m^{-1}
Vertisols, May Gassa						
0-50	2.47	21.04	2.11	0.73	9.64	2.56
50-100	2.64	19.88	2.84	0.78	10.41	2.54
100-150	2.58	19.04	3.32	0.77	10.35	2.49
Calcisols, Adi Karafiz						
0-50	0.83	13.25	1.94	0.30	5.18	1.60
50-100	1.22	15.81	3.41	0.39	5.97	2.04
100-150	1.48	14.40	3.52	0.49	7.63	1.94
Calcisols, Filiglig						
0-50	0.72	14.68	1.69	0.25	4.21	1.71
50-100	0.65	14.31	1.74	0.23	3.89	1.67
100-150	0.61	13.36	1.72	0.22	3.89	1.57
Vertisols, Dur Anbesa						
0-50	0.65	22.02	1.55	0.19	2.68	2.42
50-100	0.99	22.08	1.69	0.29	4.00	2.48
100-150	1.28	21.95	1.85	0.37	5.11	2.51
Vertisols, Gerebsegen						
0-50	0.83	21.45	2.93	0.24	3.29	2.52
50-100	1.63	19.78	3.23	0.48	6.62	2.46
100-150	2.5	24.55	4.10	0.66	8.03	3.11
Calcisols, Girat Shito						
0-50	2.7	16.14	3.15	0.87	12.28	2.20
50-100	2.85	26.98	5.29	0.71	8.12	3.51
100-150	2.78	27.79	4.87	0.69	7.85	3.54
Luvisols, Mai Haidi						
0-50	0.32	12.18	0.37	0.13	2.49	1.29
50-100	0.31	12.20	0.62	0.12	2.36	1.31
100-150	0.38	11.30	0.57	0.16	3.10	1.22
Vertisols, Gum Selasa						
0-50	0.9	20.10	1.90	0.27	3.93	2.29
50-100	0.88	20.71	1.91	0.26	3.75	2.35
100-150	1.12	19.50	2.02	0.34	4.95	2.26
Cambisols, Maidelle						
0-50	0.41	14.66	2.50	0.14	2.33	1.76
50-100	0.35	13.66	2.78	0.12	2.09	1.68
100-150	0.25	12.67	2.67	0.09	1.60	1.56
Mean±SD	1.27±0.90	17.98±4.68	2.45±1.19	0.38±0.24	5.39±2.94	2.17±0.61
Min	0.25	11.3	0.37	0.09	1.6	1.22
Max	2.85	27.7	5.29	0.87	12.28	3.54

Table 3. Anionic composition of the soils of Mekelle Plateau

Soil Depth cm	SO_4^{2-} $mmol_c l^{-1}$	$CO_3^{2-} + HCO_3^{-1}$ $mmol_c l^{-1}$	Cl^{-1} $mmol_c l^{-1}$
	Vertisols, May Gassa		
0-50	0.026	0.100	0.035
50-100	0.026	0.201	0.018
100-150	0.070	0.202	0.013
	Calcisols, Adi Karafiz		
0-50	0.052	0.304	0.014
50-100	0.052	0.201	0.005
100-150	0.035	0.203	0.004
	Calcisols, Filiglig		
0-50	0.044	0.104	0.007
50-100	0.070	0.300	0.007
100-150	0.070	0.200	0.005
	Vertisols, Dur Anbesa		
0-50	0.009	0.201	0.006
50-100	0.009	0.106	0.009
100-150	0.026	0.202	0.010
	Vertisols, Gerebsegen		
0-50	0.035	0.401	0.007
50-100	0.009	0.203	0.007
100-150	0.009	0.104	0.025
	Calcisols, Girat Shito		
0-50	0.020	0.103	0.027
50-100	0.020	0.005	0.027
100-150	0.016	0.004	0.021
	Luvisols, Mai Haidi		
0-50	0.004	0.105	0.006
50-100	0.004	0.202	0.006
100-150	0.005	0.105	0.007
	Vertisols, Gum Selasa		
0-50	0.017	0.105	0.008
50-100	0.026	0.105	0.006
100-150	0.009	0.204	0.007
Mean±SD	0.028±0.021	0.165±0.09	0.012±0.009
Min	0.004	0.004	0.004
Max	0.07	0.401	0.035

3.3 Arsenic threat

Arsenic is a semi metallic element and present in small amounts in soils and in plants and animal tissues. It occurs naturally in most soils in amounts between 1 and 70 ppm. The water soluble content is not related to the total, and may be very low in soils with high total amounts (Bear, F.E, 1964). Arsenic concentrations up to 20 ppm are quite common in soil, and up 40 ppm may be considered within the normal range (Frederic, et al., 2004). As is illustrated in Fig 3, the arsenic concentrations in soils of the Korer, Gum Selasa and Kelamino irrigated varied from 260-440, 260-300 and 260-460 ppm, respectively. In all cases arsenic is in a range serious threat as phytotoxicity in the irrigated fields of the study area.

4. Conclusions

This study revealed that in soils of the irrigated fields of the nine dams in Mekelle Plateau, Ethiopia, salinity and arsenic are building up gradually. Seemingly, increasing salinity and arsenic deposit in the soils with this trend and pace will cause phytotoxicity and eventually will impair the growth of plants. The principal quality of saline soils injurious to plants is the high osmotic pressure of the soil solution, which reduces the availability of water. Presumably, reclamation of the areas should begin now considering range of options

starting from leaching to crop selection. Crop selection can be a good management tool for these soils. Where satisfactory drainage can be economically established, leaching readily removes salt. More studies are needed to better understand the processes leading to the accumulation of arsenic in the irrigated fields of Tigray. This is important because Phytotoxicity due to As is another major concern, which requires special and more expensive remedial measures.

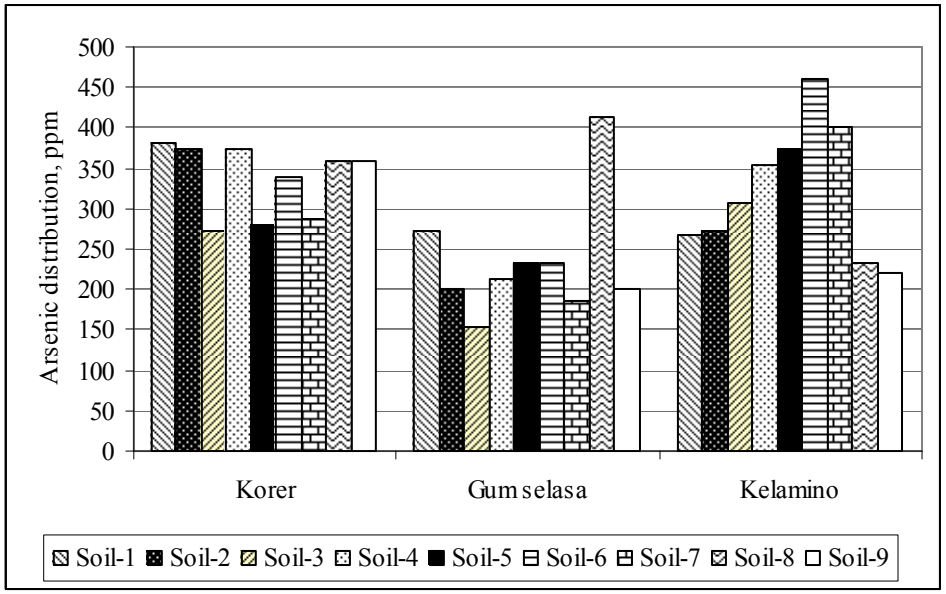

Figure. 3. Arsenic concentrations in the soils of the irrigated fields of Korer, Gum Selasa and Kelamino dams

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Isolation of Methicillin Resistant *Staphylococcus aureus* (MRSA) from AIDS Patients Attending State Specialist Hospital, Yola and Federal Medical Centre, Yola, Adamawa State, Nigeria.

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Abstract: One hundred and eleven (111) urine samples were collected from AIDS patients receiving treatment and counselling in State Specialist Hospital, Yola (SSHY) and Federal Medical Centre, Yola (FMCY). A number of organisms namely *Escherichia coli*, species of *Staphylococcus*, *Proteus, Pseudomonas, Klebsiella* and *Streptococcus* were isolated from urine samples of patients attending these two hospitals. Out of 54 isolates, 21 (39%) were identified as species of *Staphylococcus*, 12 out of these 21 isolates were identified as isolate of *S. aureus*. The prevalence of *S. aureus* among male patients was 17% whereas the respective value for the female patients was 6%. The age of the patients positive for *S. aureus* were in the range of 18-45 years. Antibiotics sensitivity tests reveals that fusidic acid has the highest percentage efficacy (66%) followed by novobiocin (58%) and methicillin (50%). So, AIDS patients may be responsible for hospital associated and community associated infection due to MRSA. [Report and Opinion- 2009; 1(6):103-107]. (ISSN: 1553-9873).

Key words*: MRSA, AIDS, methicillin, Staphylococcus sp.*

1.INTRODUCTION:

Staphylococcus aureus is the most important human *staphylococcal* pathogen and causes boils, abscesses wound infections, Pneumonia, toxic shock syndrome and other diseases. Pyomyositis is a purulent infection of skeletal muscle and is usually caused by *S. aureus*. Recently, Pyomyositis associated with HIV infections has been reported with increasing frequency in patients with or without AIDS. A recent report in Uganda stressed the importance of HIV infections as an underlying condition in patients with Pyomyositis. *Staphylococcus aureus* is the most commonly implicated organism, accounting for 90% of the cases in HIV-positive patients, 65% of the cases in HIV-seronegative patients ($P<0.01$), and more than 90% of the recovered organisms in the tropics. Other bacteria were found less frequently in HIV-positive patients and included *Streptococcus* group *Streptococcus spp*, *Salmonella enteridis, Salmonella spp, E.coli, Citrobacter freudii, Morganella morganii* and *Pseudomonas aeruginosa*. (Ansaloni *et al*, 1996).

Recently strains of multiple drug resistant *S. aureus* have appeared and proven very difficult to treat (Prescott, 2005). During the late 1950s and early 1960s, *Staphylococcus aureus* caused considerable morbidity and mortality as a nosocomial or hospital acquired pathogen and has become the head leading cause of nosocomial infection during the last 2 decades (Nimmo and Phyford, 2003). Since then penicillinase resistant semi synthetic penicillin as proved to be successful antimicrobial agents in the treatment of staphylococcus infections. Unfortunately, MRSA (methicillin resistant *Staphylococcus aureus*) strains isolated are on increasing resistant to multiple non-β-lactam containing antimicrobial drugs. Recent report of vancomycin-resistant *S.aureus* fore shows an area of chemotherapy in which effective bactericidal drugs to treat infections with this organism may not be readily available, (Cookson, 2002).

MRSA is a problematic pathogen in human medicine and appears to be emerging in the world. Historically, hospital associated MRSA infections have predominated in humans and contributed to significant illness and death. Recently a shift in the epidemiology of MRSA infections have been documented, where by community associated methicillin-resistant *S.aureus* (CA-MRSA) infections have become more common. CA-MRSA may arise from the hospital origin clone that are carried into the community and then transmitted between the communities and then transmitted between or from de novo development of resistance through acquisition of resistance factor (mec A) by methicillin sensitive strains of *S. aureus*

2. MATERIALS AND METHODS:

2.1 *SELECTION OF HOSPITALS*

The hospitals selected for this study were State Specialist Hospital, Yola (SSHY) and Federal Medical Center, Yola (FMCY). The patients diagnosed with AIDS in Adamawa state are generally referred to these two hospitals for treatment and counseling purpose. They collected their antiviral drugs at reduced cost from FMCY. The terminal ill patients are generally advised to get admission in either of these two hospitals and they are generally referred to Microbiology laboratories for routine microbiological analysis

2.2 *PERIOD OF STUDY*

The period of collection of sample was between the month of April, 2007 and May, 2007.

2.3 *SPECIMEN COLLECTION*

In the clinic, the counseling unit, the wards and hospital laboratories, urine samples were collected from the patients after explaining the aim and objectives of the research to them. Only the patients who were willing to take part in the research selected for this work. Sterile screw-caped wide – naked specimen bottles were used to collect urine samples with the help of the staff of clinic, counseling unit, the wards and the hospital laboratories. All the samples were properly labeled for each sample, age and sex were recorded. The samples after collection were transported to the microbiological laboratory of SSHY within 2-3 hours of collection using a cooler packed with ice blocks.

2.4 *ISOLATION OF ORGANISMS*

A loopful of each specimen was inoculated onto MacConkey agar using streak plate method. Then the plates were incubated at 37^0C for 24 hours. The discrete colonies were isolated and further subcultured using MacConkey agar and were kept at 4^0C for further research work (Benson, 2002). Same procedure was followed for isolation of discrete colonies using CLED agar and mannitol salt agar from the collected urine samples.

2.5 *IDENTIFICATION OF ISOLATES*

The following procedures were used in order to identify the isolated colonies on MacConkey agar, CLED agar and Mannitol salt agar.

2.6 *MICROSCOPIC CHARACTERISTIC OF ISOLATED COLONIES*

The colour and size of the colonies were recorded for identification purpose (Cheesbrough, 2002).

2.7 *GRAM STAINING*

*G*ram staining was done according to the method stated in Cheesbrough, 2002.
Twenty one isolates which were considered to be *S. aureus* isolates were selected for further studies.

2.8 *COAGULASE TEST*

A loopful of growth for each isolate was inoculated into 5ml of peptone water which was then incubated at 37^OC until turbidity reached 0.5 Mcferland standards. Then test tube procedure and slide test method were employed using this broth culture (Benson, 2002).

2.9 *CATALASE TEST*

A drop of hydrogen peroxide (3%) was placed on a microscopic slide and a bit of growth of the test organisam was placed and then emulsified. Effervescence caused by liberation of oxygen as gas bubbles indicates the presence of catalase in culture under test (Benson, 2002).

2.10 *SUGAR UTILIZATION TEST*

The sugars used were glucose, lactose and sucrose for the twenty one isolates obtained. A loopful of growth for each isolate was inoculated into 5ml of peptone water which was then incubated at 37^0C for 24hours. 1ml of 1% sterile sugar solution was added to a tube containing Durham tube (inverted) and to it 1ml of cell suspension as prepared above was added. Observations were made for any color change and gas production after 24hours.

2.11 *NOBOVIOCIN TESTING*

*T*he isolates were tested against Novobiocin (5µg/ml) in order to observe their sensitivity or resistance against this antibiotic. Streak plate method was used for this purpose (Benson, 2002).

2.12 *SENSITIVITY TEST*

Twelve *S.aureus* isolates were obtained after biochemical tests. These were used for this purpose. A speck of growth of each isolate was inoculated into sterile peptone water incubated for 24 h and 1ml of the growth using sterile spreader was evenly spread on the surface of nutrient agar plate. Sensitivity discs containing conventional antibiotics manufactured by Mastering Laboratories Ltd. placed on surface of nutrient agar plate and incubated at 37^oC for 24 h. The antibiogram was then read and recorded (diameter of zone of inhibition). In this study, zone of inhibition less than 3mm was considered as resistant (Cheesbrough, 2002)

Table 1: Results of Gram staining, biochemical test and Novobiocin test

Isolate No.	SC	TC	Catalase	Sugar	GS	Novobiocin	Novobiocin
A_1	-	-	-	+	+	S	-
A_2	+	+	+	+	+	"	+
A_3	+	+	+	+	+	"	+
A_4	-	-	-	+	+	"	-
A_5	-	-	-	+	+	"	-
A_6	+	+	+	+	+	"	+
A_7	+	+	+	+	+	"	+
A_8	-	-	-	+	+	"	-
A_9	+	+	+	+	+	"	+
A_{10}	+	+	+	+	+	"	+
A_{11}	-	-	-	+	+	"	-
A_{12}	+	+	+	+	+	"	+
A_{13}	-	-	-	+	+	"	-
A_{14}	+	+	+	+	+	"	+
A_{15}	-	-	-	+	+	"	-
A_{16}	+	+	+	+	+	"	+
A_{17}	+	+	+	+	+	"	+
A_{18}	-	-	-	+	+	"	-
A_{19}	+	+	+	+	+	"	+
A_{20}	-	-	-	+	+	"	-
A_{21}	+	+	+	+	+	"	+

KEY: + = Positive, - = Negative, S C = Slide Coagulase, T C= Tube Coagulase,
G S = Gram staining, S = Sensitivity

Table 2: Showing prevalence of organisms among studied groups in relation to age and gender from different locations.

Location	Age	Total no. of sample collected	Total (%) of *Staphylococcus* sp.	Total (%) of *S. aureus*	Total (%) of other organisms
FW	20 – 52	12	1 (4.76)	-	6(14.3)
MW	23 – 44	9	3 (14.28)	2 (16.7)	3 (7.1)
CUF	19 – 34	5	-	-	3 (7.1)
CUM	24 – 37	8	1 (4.76)	1 (8.33)	4 (9.2
HLF	5 – 6	6	1 (4.76)	-	2(\4.8)
FMCF	7 – 37	36	8 (42.85)	4 (33.33)	3 (3.1)
FMCM	4 – 45	38	5 (23.80)	4 (33.33)	11(26.1)
HLM	26 – 29	3	1 (4.76)	1 (8.33)	)12 (2.4)

KEY:-
FW - female warder
MW - melwe warder
CUF -counseling unit female
CUM -counseling unit male
HLF -hospital laboratory female, sshy
HLM -hospital laboratory male, sshy
FMCF -federal medical center female
FMCM- federal medical center male

Table 3: Showing percentage Efficiency of various antibiotics

Antibiotics	No. of sensitive *S.aureus*	% Efficacies
Chloramphenical	2	16
Erythromycin	2	16
Fusidic acid	8	66
Methicillin	6	50
Novobiocin	7	58
Penicillin G	-	0
Streptomycin	1	8
Tetracycline	-	0

KEY: - Negative

3. RESULTS AND DISCUSSION

Out of one hundred and eleven urine samples analyzed, sixty one were collected from female patients, forty three were collected from male patients and seventy seven were collected from children suffering from AIDS. Fifty four (54) colonies were isolated from CLED agar, Mannitol salt agar and MacConkey salt plates and these were properly labeled. Twenty one (21) isolates showed cultural characteristics of *Staphylococcus sp.* whereas other isolates were *E.coli* and species of *Proteus, Pseudomonas, Klebsiella* and *Streptococcus*. Results of Gram Staining experiments reveal that all the twenty one isolates (A1-A21) were gram positive (Table 1). Results of biochemical characteristics of isolates reveals that out of 21 isolates 12 were *S.aureus (Table 1),* All the isolates were sensitive to Novobiocin (Table **1).**

Al-Tawfiq et al (2000) reported that pyomyositis, a purulent infection of skeletal muscles and usually caused by *S.aureus*, associated with HIV infections with increasing frequency in patients with or without AIDS. Also Herchline, 2007 observed that the major infections related to AIDS patients include urinary tract, skin infections, pneumonia, salmonella septicemia, pyomyositis and mycobacterium tuberculosis. The organisms isolated from the patients were responsible for some of these infections.

Schwartzman *et al.* (1991) reported that *S.aureus* is most commonly implicated organism in HIV positive patients and other bacteria present in HIV positive patients include *Streptococcus* group *C, Streptocossus spp, S.enteritidis, Salmonella spp, Escherichia coli, Citrobacter ferundi, Morganella morganii* and *Pseudomonas aeroginos*a. Results of Novobiocin

Table 2 shows the prevalence of organisms among the studied group in relation to age and gender. The prevalence of *S.aureus* among the male patients was 17% whereas the prevalence among female patients was 6%. It may be due to the fact that male patients often still have multiple sex partners contrary to female patients with exception of female sex workers and are at risk of having different types of infection. Out of 111 samples only 12 positive for *S.aureus* (10.8%). The ages of the patients' positive for S.aureus were in the range of 18-45years. The patients of this age group are engaged in sexual activities and most of the times suffer from UTI, skin infection and pneumonia (Madigan et al, 2000).

The *Staphylococcus aureus* isolates were selected for sensitivity testing against some conventional used antibiotics. The percentage efficacy of various antibiotics are shown in Table 3.Out of those 12 *S.aureus* isolates, only 6 were methicilline resistant. The result for determination of percentage efficacy of various conventional antibiotics show that fusidic acid has the highest antimicrobial activity with percentage efficacy of 66% followed by novobiosin (58%) and methicillin (50%). The results obtained from this study revealed that AIDS patients may be responsible for hospital and community acquired infection caused by MRSA.

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