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Relationship of Biometric Size-Weight, Nutritive Value, and Metal Concentrations in *Clarias lazera* (Cuvier and Valenciennes) Reared in Treated Wastewater

Manal M. A. Awad Elkareem[1], Abeer M. H. Karrar[2] and Abdel Karim S. Ali[3,*]

[1]Department of Biology and Biotechnologies, Faculty of Science and Technology, Al Neelain University; [2]Environment and Natural Resources Research Institute, National Center for Research, Ministry of Science and Communication, [3]Department of Environmental Sciences, Faculty of Science and Technology, Al Neelain University, Khartoum, P.O. Box 12702, Sudan

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Abstract

The objective of the present study was to investigate the nutritive value and heavy metals accumulation in the flesh of *Clarias lazera* (Cuvier and Valenciennes) reared in the discharge canal of Soba wastewater treatment station, south Khartoum, Sudan. A total of 57 fish were collected from the canal and 52 from the White Nile which served as the control. The proximate composition of fish and concentrations of eight hazardous heavy metals i.e. chromium (Cr), iron (Fe), copper (Cu), zinc (Zn), lead (Pb), robidium (Rb), strontium (Sr), and mercury (Hg) in the flesh of *C. lazera* were measured. The results which were statistically analyzed revealed insignificant differences in moisture, ash, fat contents, energy value, and fat: protein ratio (P>0.05) between the two studied sites. A significant difference (P<0.05) was evident in the protein content of fishes from both locations. Heavy metals accumulation in *Clarias* tissues differ from one element to another depending on each element characteristics and local environmental conditions e.g. Sr was higher in the White Nile fishes than in treated wastewater fishes (P<0.05). Accumulation of Pb and Hg was comparable in wastewater and White Nile (P>0.05). Fe, Cu, Zn and Rb concentrations were significantly higher in treated wastewater fishes than natural water fishes (P<0.05). Cr was also higher in treated wastewater fishes (P>0.05). Nevertheless, the concentrations of most considered elements were lower than levels recommended by various international agencies.

Key words:*Clarias lazera*, Heavy metals, Accumulation, White Nile, Treated wastewater.

1. Introduction

Treated wastewater was used in 19th century in Europe to irrigate crops (Ensink and van der Hoek, 2007). Reuse of treated wastewater for fish aquaculture is practiced in many countries including India (Bunting, 2006), Egypt (Misheloff, 2010), and Netherland (Oberdieck and Verreth 2009). As water demand becomes an increasingly important concern in many places, and especially essential for the increasing human population, fish farming falls within the many options which exist for productive wastewater treatment design systems (WHO, 2006). Popular fish species suitable for fish farming in treated wastewater include catfish, tilapia spp. trout, carp, and many others. The annual production depends on fish species, local and environmental conditions. According to Girard (2011), there are many constraints to reusing treated wastewater for rearing fish, such as lack of knowledge, limited available sites; rapid urbanization, rapid eutrophication, improved sanitation, rapid industrialization contamination, social and cultural acceptance and climate. The pathogen transmission risk through treated wastewater fish farming represents a controllable risk; i.e., pathogen loads can be reduced to acceptable levels if adequate measures are adopted (Straus 1996). Microbial requirements for waste-fed aqua cultural schemes should be compatible with background levels in natural waters, since the harvesting of fish and other aquatic animals is generally unrestricted and socially accepted.

Fish is one of the most important available sources of animal protein in the tropics, and has been widely accepted as a good source of protein and other elements for the maintenance of a healthy body (Tidewell and Allan, 2001). Heavy metals are persistent contaminants in the environment that come to the forefront of dangerous substances such as cadmium, lead, mercury, copper and zinc that cause serious health hazards to humans and animals (Ahmed *et al.*, 1998). The agricultural and

* Corresponding author. e-mail: aksabirali@gmail.com.

industrial wastes, partially treated or untreated regularly, are being discharged into surface water (Forstner and Wittmann, 2007). Heavy metals present in such polluted waters are absorbed through gills, skin and digestive tract of fish by bio-concentration and bio-magnification. Heavy metals are natural trace components of the aquatic environment, but their levels are increased due to domestic, industrial, mining and agricultural activities (Mance, 1987; Kalay and Canli 2000). At low levels, some heavy metals such as copper, cobalt, zinc, iron and manganese are essential for enzymatic activity and many biological processes. However, the essential metals may also become toxic at high concentrations (Bryan 1976 and Authman *et al.,* 2012). Other metals, such as Cd, Hg, Pb play unknown essential roles in living organisms, and are toxic at even low concentrations (Authman *et al.,* 2013).

Clarias lazera (Cuvier. and Valenciennes) is a freshwater fish which attracts attention as a potential fish for aquaculture (Babiker, 1984). According to Chervinski (1984), presence of a breathing apparatus enables the fish to withstand low level of oxygen and a wide range of temperatures. It is an omnivorous feeder found mainly in shallow waters; young ones feed on ostracods and aquatic insects while adults feed on any potential food like zooplankton and molluscs, but mainly on fish e.g. *Oreochromis niloticus* (Amirthalingam and Khalifa, 1965).

The aim of this study was to highlight the implications of the reuse of treated wastewater for fish culture, and to examine and compare the levels of toxic heavy metals accumulation in the muscle tissue of *C. lazera* from the polluted discharge canal of Soba wastewater treatment station and non-polluted of Jebel Aulia Reservoir on the White Nile, south Khartoum, Sudan. The proximate chemical composition and condition factor of fish were studied as complementary aspects. The present study is expected to shed light on the importance of the reuse of treated wastewater for fish farming and its promising role in contributing to provide a safe food for the increasing Sudanese population.

2. Materials and Methods

A total of 57 specimens of *C. lazera* (Arabic Garmouth) were collected from the effluent (discharge) canal carrying the treated wastewater from the maturation ponds, in addition to 52 specimens from the White Nile in the vicinity of Jebel Aulia Dam (control). Fish samples were collected from local fishermen during the period April 2012-April 2013. The canal (Figure 1) is located in the Southern part of Khartoum State ca. 15 Km, (15°29' 55"; 15°30' 25" N; 32°32' 36" – 32°36' 06" E).

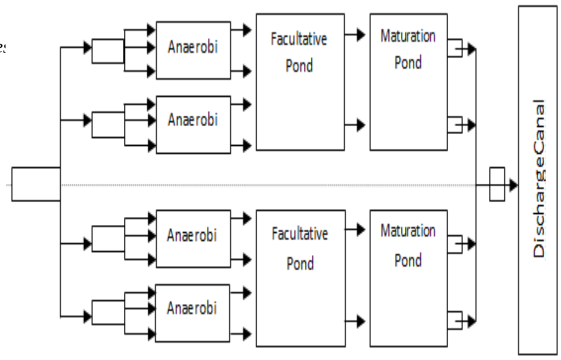

Figure 1: Ponds arrangement, flow pattern and the effluent discharge canal in Soba Wastewater Stabilization Station (Source: Ali and Hag Ibrahim, 2005).

The 2nd site, Jebel Aulia dam on the White Nile, was built in 1937 ca. 50 Km south of Khartoum to store $3.5X10m^9$ of water (Rzoska *et al.* 1955). The dam length is 5 km and creates a large lake with a width ranging between 1-4 km and a maximum depth 22.5 m. As a result of the dam, a large shallow lake of about 12000 hectares was formed capable of storing 3.5 milliard m^3. The maximum depth of the reservoir is ca. 15 m attained during high flood (August-mid September).

For each fish specimen, the total and standard lengths were measured, and then each fish was weighed. The condition factor (K) was calculated according to Le-Cren, 1951 equation:

$$K = 100.W/L^3$$

W is fish body weight (g) and L is fish total length (cm)

Fishes were sexed and aged using vertebrae. Use of vertebrae for age determination was proved to be one of the reliable structures for fish ageing (Bishai and Abu Gideiri 1965, Mishrigi 1967, Bishai 1970, Gumaa 1974 and Tweddle 1975). Otolith was not used for aging *C. lazera* because its major advantage over other tissues e. g. scales and vertebrae is the presence of clearly visible daily lines (Pannella 1974). In addition, preparation of otoliths for light microscopy or scanning microscope (Liew 1974) is rather difficult and requires facilities not available in our institutes. Sexual maturity was determined according to Nikoliskii (1963). The stomach food content was investigated using the method described by Hynes (1950) where stomachs contents are examined and the individual food organisms was sorted and identified. The number of stomachs in which each item occurs is recorded and expressed as a percentage of the total number of stomachs examined. The collected fish were then skinned; the flesh was taken from different sites of the body to make sure that the examined sample is well representative to the whole body. Flesh samples were kept in air-tight plastic bags and frozen at $0.0\text{-}5.0^0C$ till they were used for moisture and ash contents determination.

The frozen samples were then freeze-dried for 24 hrs using (Edwards High Vacuum 2507 Freeze Dryer). The dried samples were ground using a non metallic mortar. Powder samples were then kept in the air-tight plastic bags. All chemical constituents were determined according to Pearson 1976 and AOAC 1980. These include moisture; protein, fat and ash contents. Protein was evaluated by Micro-Kjeldahl Method; fat content by using soxhelt extraction method. The energy value was calculated from the fat and protein contents of samples using the values 9.02 Kcal./gm for fat content and 4.27 Kcal./gm for protein content as recommended by FAO (1989).

X-ray fluorescence spectroscopy (XRF) was used for qualitative and quantitative determination of heavy metals (Tertian and Claisse 1982). These are namely: Cr, Fe, Cu, Zn, Pb, Rb, Sr and Hg. According to Talbot (1987), XRF has the potential and capacity to give accurate linear response to a broad spectrum of elements in approximately 0.0-500 mg/Kg-1.

The data obtained was statistically analyzed using SPSS package (t-test for normal data and Mann Whitney u test for data not normally distributed).

3. Results and Discussion

The results of the present study (Table 1) showed greater sizes and weights of fishes in treated wastewater compared to those collected from the White Nile ($P<0.05$). The same applies to standard lengths although the difference was insignificant ($P>0.05$). According to Mason 1991, such results are possibly attributed to the availability of food in treated wastewater and the presence of organic matter which is rich in proteins, carbohydrates, and fats.

Table 1: Weights and standard lengths of *C. lazera* in treated wastewater and White Nile

Parameter	Treated Wastewater	White Nile	*P*
Weight (gm)	528.96±38.9	360±45.90	*
Standard length	35.04±1.14	30.94±1.21	NS

The condition factor of fish reared in both study sites was calculated to evaluate fish situation. The mean condition factor of fish was found to be 1.23 g/cm^3 and 1.22 g/cm^3 in treated wastewater and White Nile, respectively. Both values were above 1.0 indicating good conditions of the fish (Barnham and Baxter 1998), however, they were below those obtained by Nwabueze 2013 for *Clarias anguillaris*. Furthermore, King (1995) attributed differences in condition factors of fish to food abundance, adaptation to the environment and gonadal development. According to Aloo (1999), the intensity of infection with *Contracaecum* was increased with the increase in the size of bass *Micropterus salmoides*, from Lake Naivasha and the Oloidien Bay, Kenya

Only two stages “I and IV” were observed out of Nikoliskii (1963) six stages for sexual maturity (Stage 1, Immature; Stage 11, quiescent; Stage 111, maturing: Stage 1V, mature: Stage V running; Stage V1, Spent).

Table 2 illustrates the difference in body weight according to sex, age and maturity stage and the results gave an insignificant difference with sex and maturity stage ($P>0.5$) in both localities. However, weights of mature fish were higher than immature fish. Regarding age, a significant difference was encountered ($P<0.05$) in weights of fish; older fish weights were higher than younger ones in both sites.

Figure 2 shows the moisture, ash, fat and protein contents which were examined to assess the fish flesh quality. The fat/protein ratio was calculated to indicate the nutritive value of flesh. Protein content in wastewater fish was higher than in the White Nile ($P<0.05$). Fat, moisture content and fat/protein ratio were almost similar ($P>0.05$) in the two sites (0.212 for Treated wastewater and 0.217 for the White Nile).

The food value of fish is normally estimated as the percentage of the edible portion to the total weight of fish and its contents of the basic nutrients i.e. fats and proteins (Karrar, 1997). In the present study, the energy values calculated were 0.0683 and 0.0629 for wastewater and White Nile fish, respectively. These figures indicate that the nutritive values of the fish from both study sites are comparable; however, freshwater fishes are slightly more nutritious due to their low fat/protein ratio.

Table 2: Variation in body weights of *C. lazera* in treated wastewater and White Nile according to sex, age group and maturity stage

	Sex		Age Groups				Maturity stage		
	Treated wastewater	White Nile	Treated wastewater		White Nile		Treated Wastewater		White Nile
Male	459.38± 63.5	392.00±86.60	1	427.20±29.8	1	308.60±29.30	A	371.04±33.5	336.8±58.7
Female	575.35+63.50	333.28±42.20	2	936.03±53.1	2	1480.00±10.0	B	714.40±53.7	426.4±10.0
P	NS	NS	*		*		*		*

*significant; NS insignificant

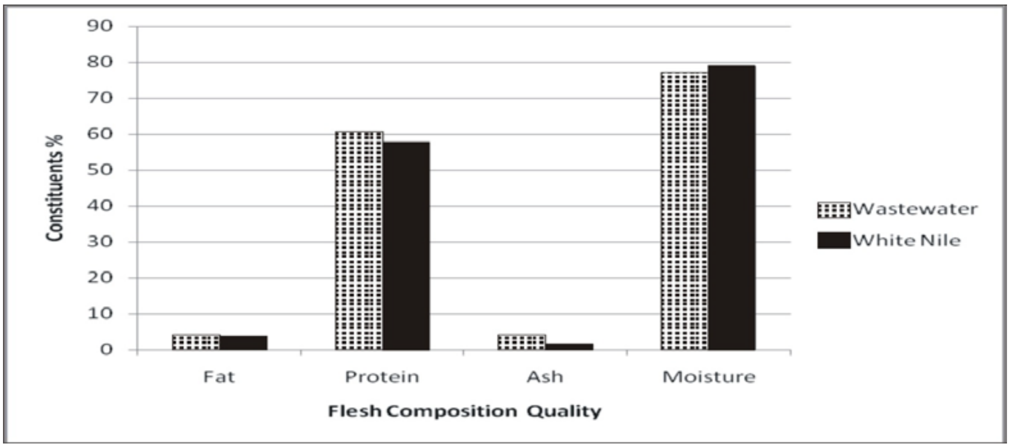

Figure2. Chemical composition of *C. lazera* in treated wastewater and White Nile

The energy value was higher in the White Nile fish (445.911+17.23 Kcal / 100 gm) than in wastewater (414.540±8.394 Kcal / 100 gm), but the difference was insignificant (P>0.05).

Figures 3a, 3b and 3c illustrate no significant difference in fat, protein and ash contents with sex, age and maturity stage, respectively (P>0.05). Exceptions were encountered in females' fat content in the White Nile that showed significantly higher values than in males (P<0.05).

Proximate composition showed no significant variation according to sex in both localities. This finding is in agreement with Mohammed *et al*. (1988) who assessed the chemical composition of *Mugil cephalus* along the Sudanese Red Sea Coast. Also proximate composition showed no significant variation with age. That result was in contrast to Dambergs (1963), but older fish reflected slight insignificant increase in fat and protein contents.

Protein and ash contents were insignificantly higher in mature fish in both study sites and that was also in contrast to Dambergs (1963), but fat content showed insignificantly higher levels in the immature fish. According to Hoar (1957), fat storage in the muscle tissue increased prior gonads maturation to provide energy for spawning activities.

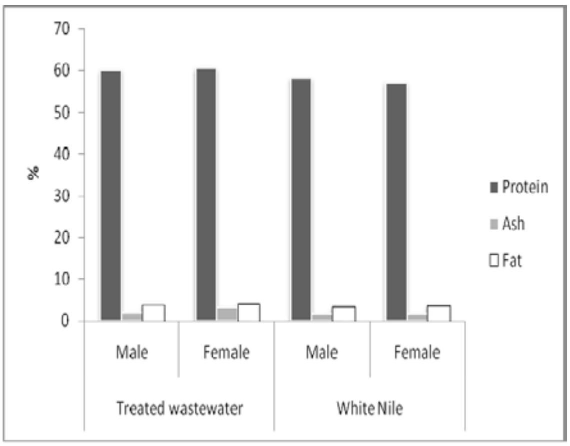

Figure 3a. Variation in Chemical composition of *C. lazera* in wastewater effluent canal and White Nile according to sex

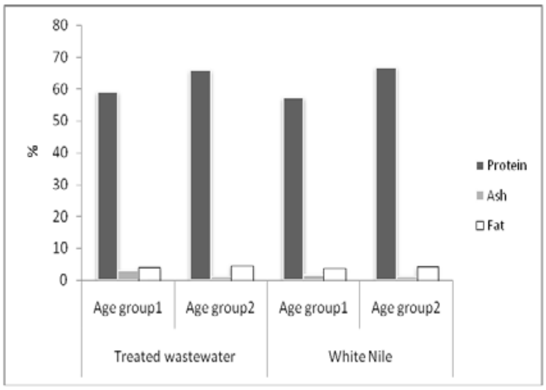

Figure 3b. Variation in Chemical composition of *C. lazera* in wastewater effluent canal and White Nile according to age group (age group 1: 1, 2 and 3 years; Age group 2: 4 and 5 years)

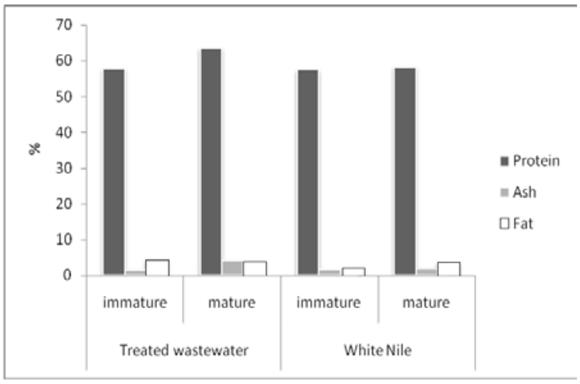

Figure 3c. Variation in Chemical composition of *C. lazera* in wastewater effluent canal and White Nile according to age

Concentrations of Fe, Cu, Zn and Rb (Table 3) were significantly higher in treated wastewater fishes than natural water fishes (P<0.05). In view of this, they were further analyzed with sex, age group, and maturity stage (Figures4a, 4b, 4c). Fe and Zn showed no variation with all the previously mentioned parameters in both study sites (P>0.05). Cr was also higher in treated wastewater fishes (P>0.05). Hg and Pb concentration was higher in treated wastewater than the White Nile. Statistically, the two heavy metal expressed insignificant difference ((P>0.05). Sr was significantly higher in the White Nile compared with treated wastewater (P<0.05).

Table 3. Heavy metals concentrations in *C. lazera* in treated wastewater and White Nile

Conc. (ppm)	Wastewater	White Nile	*P*	Permissible limits (µg/g wet weight)
Cu	4.86± 0.56	1.26± 0.16	*	3.280 "IAEA 2003; FAO 1983a"; 20 "MAAF 2000"
Rb	177.63± 23.18	12.89± 1.54	*	2.86 "IAEA 2003"
Cr	0.05± 0.02	0.03±0.01	NS	0.730 "IAEA 2003"
Fe	72.64± 12.60	6.94±0.93	*	146 "IAEA 2003"
Zn	497.20± 44.7	37.83±7.04	*	30 FAO 1983a; 40 FAO/WHO 1989
Hg	60.03 ± 1.30	57.42±3.32	*	0.222 "IAEA 2003"; 0.50 "EC 2006"
Pb	0.17± 0.02	0.09±0.01	*	0.2 "EC 2005"
Sr	2.7 3 ± 2.46	17.55 ± 10.35	*	130 "IAEA 2003"

These results are in agreement with the findings of many authors e.g. Clement and Lovel (1994) and Gomez (2011). However, ash content was significantly higher in treated wastewater fishes (*P*<0.05). The concentrations of most elements considered in this study were lower than levels recommended by the international agencies e.g.EC (2006); EC (2005); IAEA (2003); FAO/WHO (1989); FAO (1983a). Rb showed significant variation with sex and age in both study sites but not with maturity stage. Cu concentration showed no variation with sex in both study sites (*P*>0.05). However, it gave significant differences with age in the two studied sites. With maturity stage, Cu was much higher in treated wastewater fish and expressing a significant difference (*P*<0.05).

Treated wastewater fish were found to accumulate considerable amounts of heavy metals compared with White Nile fish. This is likely because metals have certain properties which make them difficult to treat or remove from wastewater if not treated by certain chemical methods (Mubarak, 2014), and living organisms usually form an intimate relationship with the chemical composition of their environment (Elgobashy *et al.* 2001). According to Jezierska and Witeska (2006), fish living in polluted waters tend to accumulate heavy metals in their tissues. Generally, accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). Concentrations of Pb and Hg in treated wastewater fish were almost similar to fish of natural non-polluted water possibly because surface water does not always reflect the real situation of heavy metals pollution. This is expected since the physico-chemical characteristics of water normally affect the distribution of metals in water. In addition, Karrar (1997) reported that some metals have the property of being rapidly adsorbed to particulate materials like detritus and suspended sediments. Moreover, some biological processes like uptake by planktons through assimilation can exert the same effect (Abu Gideiri, 1980).

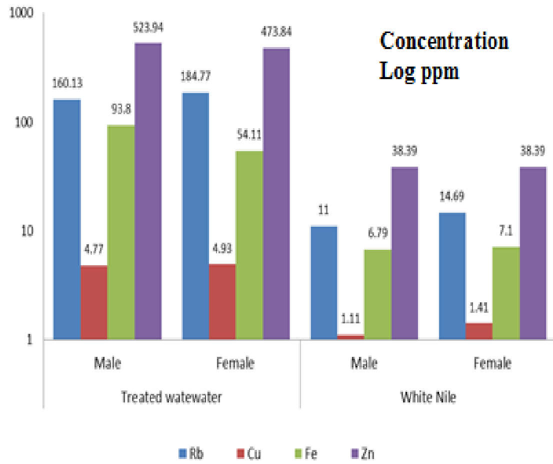

Figure 4a. Heavy metals accumulation in *C. lazera* in wastewater effluent canal and White Nile according to sex

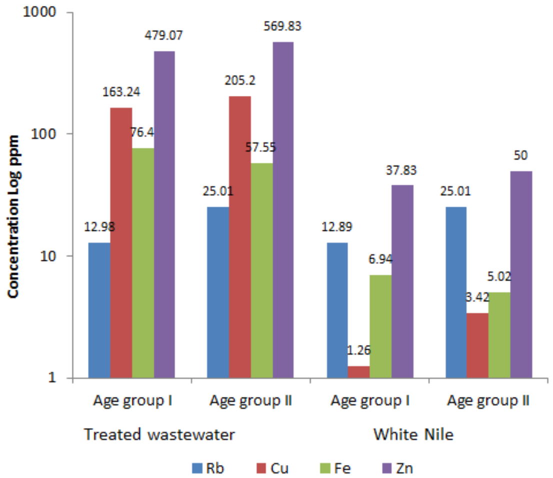

Figure 4b. Heavy metals accumulation in *C. lazera* in wastewater effluent canal and White Nile according to age

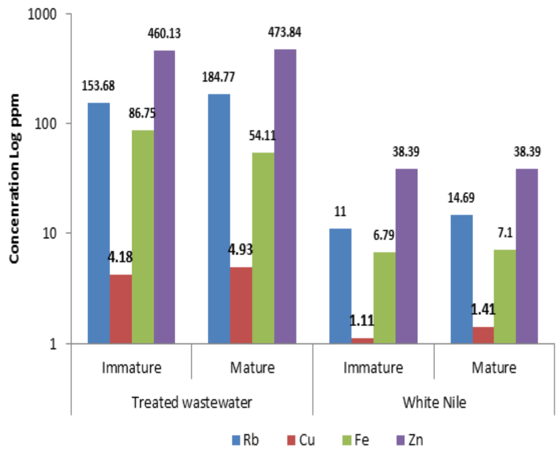

Figure 4c. Heavy metals accumulation in *C. lazera* in wastewater effluent canal and White Nile according to maturity stage

Cu, Rb, Fe and Zn showed significantly higher levels of accumulation in fish of treated wastewater than White Nile fish (*P*<0.05) probably because their concentration was lower in the latter site. However, except for Zn, the concentrations of other metals did not exceed the permitted international levels (FAO 1983a, b; Huss, 1994; Al-Wher, 2008). Cr, Hg and Pb reached the wastewater treatment plant with the wastewater transported from the Khartoum Industrial Area e.g. Khartoum Tannery, Printing facilities, paints factories and other industrial activities (Sabir *et al.* 2007). These metals were not subject to any biodegradation since the station depends mainly on natural micro-organisms which can only decompose organic matter. Zn, Fe and Cu concentrations were higher than Hg, Cr and Pb in both sites. Such situation could be attributed to the fact that Zn, Fe and Cu are essential micro-nutrients required in life processes, so most organisms have the ability to keep them at high concentrations in their bodies. This capacity is enhanced by certain feeding and metabolic processes which lead to high accumulation. Furthermore, many of these metals are capable of forming complexes with the available organic substances and hence have the tendency to be fixed in tissues rather than being excreted (Mara and Cairncross 1989). Deposition of heavy metals in fish tissues in treated wastewater seems to inflict no harmful effects on *Clarias* which survive successfully with increasing numbers and sizes. In conformity with this, Zaki (2007) stated that aquatic fauna are exposed to chronic substances i.e. pollutants that do not cause heavy mortalities but fishes survive and accumulate various amounts of microbial or chemical residues of heavy metals which might result, in extreme cases, in unpleasant tastes or are potentially dangerous. Saeed (2007) results on *C. gariepinus* in Lake Edku, Egypt, revealed that accumulation of heavy metals generally associated with specific tissues/organs of the fish e.g. Cu in liver, Cd in gills, liver and ovary and kidney and Pb in gills and ovary. Furthermore, Benamar and Zitouni (2013) reported that accumulation of Cu is higher than Cr in liver tissue compared with the muscle tissue of *Sardinella aurita* collected from Oran Coastline in Algeria. Das and Gupta (2013) concluded that Cu accumulation pattern in the Indian flying barb *Esomus danricus* was related to metal concentration and increased with exposure time. The findings of this study are also in agreement with Mason (1991) and Ebrahimi *et al.* (2007) who reported that consumption of edible tissue of mullet which is subjected to heavy metals pollution is not harmful to humans and that the heavy metals accumulation in fish tissues is below the Egyptian standards. Presence of some organic substances in treated wastewater may render heavy metals less toxic than they would be in pure uncontaminated conditions. The form of heavy metal to which aquatic organisms are exposed is important and determines its overall toxicity (Singh *et al.* 2011).

Cu, Rb, Fe and Zn showed slightly different pattern when analyzed according to sex. Values of Fe, Cu, and Zn showed insignificant variation between males and females in both study sites, while Rb showed significant increase in female specimens from both study areas which may be due to the high ash content of females. Patin (1982) however, reported a positive correlation between the ash content of the fish and its content of a certain metal. Authman and Abbass (2007) findings on heavy metals in *Tilapia zilli* and *Mugil cephalus* tissues indicated that accumulation was much higher than concentration of these heavy metals in their surrounding waters. The water characteristics for the two sites, obtained by Sabir *et al.* (2007) were in conformity with the results of the present study.

According to age, Cu, and Rb showed significantly higher accumulation in older fish in both localities, Zn insignificantly showed the same (Figure 4b). That is likely because fish accumulate elements throughout their life, so older fish would contain more of these substances (Connel 1975; Huss 1994). The previous finding is in agreement with Patin (1982) who found positive correlation between the actual metal content of a fish and its age, size and weight. Fe although showed no variation with age, the difference was not significant.

The pattern of variation of Cu, Rb, Fe and Zn with the stages of maturity of fish from both study sites did not follow certain models although Cu, and Rb showed insignificantly higher levels in mature fish in both study sites and Zn showed the same in treated waste water fish. According to Huss (1994), fish becomes mature when it reaches certain size not a certain age so mature fish with larger sizes are expected to contain more heavy metals as the actual metal content of a fish is positively related to its size and weight.

Insects, phytoplankton, zooplankton, weeds, molluscs and fish parts were detected as major food constituents in the stomach of *Clarias lazera* from both studied localities (Figure 5). Phytoplankton, fish parts and molluscs showed significant variations between the two sites whereas mean values of insects, zooplankton and weeds were almost similar ($P>0.05$). Fish, molluscs and zooplankton were much more detected in stomachs of freshwater fish because wastewater doesn't suit their growth. According to Jumaa (1974) the partially low oxygen, elevated CO_2 and the relatively high concentrations of nitrogen and nitrates create unfavorable conditions for zooplanktons growth. Besides, metals in both cationic and soluble complex forms can be toxic or inhibit zooplanktons growth (Biesinger and Christenser 1972). Superiority of phytoplankton and insects can be explained as due to presence of abundant organic matter in the treated wastewater which furnishes suitable conditions for their growth.

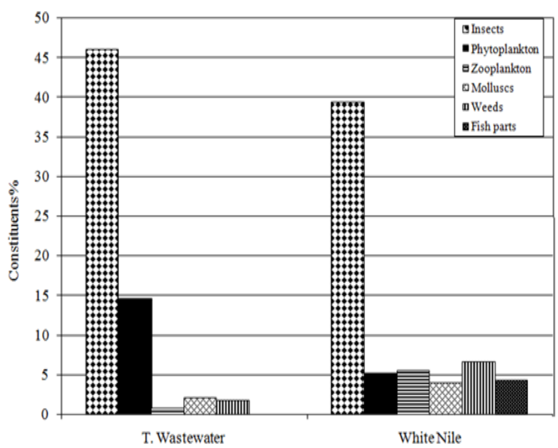

Figure 5. Food composition of *C. lazera* in treated wastewater effluent canal and White Nile

4. Conclusions

Insignificant variations were evident in proximate chemical composition of fish with age, sex, and maturity stages in both sites. However, fish collected from the treated effluent canal expressed higher sizes; weights and protein content are preferred by local consumers. Nutritive value of fishes reared in treated wastewater effluent was high and almost similar to that of natural habitat fish with significantly higher protein content. In spite of possible cultural bias against fish reared in treated wastewater effluents, the average concentrations of most hazardous heavy metals e.g. Hg and Pb are comparable to those obtained from fishes of the White Nile and are well below the values recommended by IAEA (2003), EC (2006) and other relevant agencies for human consumption. Zn concentrations exceeded the recommended level set by international agencies for human consumption and may constitute a potential health risk if ingested in large quantities.

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