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Research Note / Araştırma Notu

Comparative Study of some Haematological Parameters in European Eel (*Anguilla anguilla* L., 1758) Caught from Different Regions of Ceyhan River (Adana, Turkey)

*Aysel Şahan[1], Tülay Altun[1], Fatma Çevik[1], İbrahim Cengizler[1], Erdal Nevşat[2], Ercüment Genç[3]

[1]*Cukurova University, Faculty of Fisheries, Department of Aquaculture, Turkey, 01330*
[2]*The VI[th] Regional Directorate of the State Hydraulic Works (DSI), Turkey*
[3]*Mustafa Kemal University, Faculty of Fisheries, Department of Aquaculture, Turkey*
**E mail: ayaz@cu.edu.tr*

Özet: ***Ceyhan Nehri (Adana, Türkiye)'nin farklı bölgelerinden yakalanan avrupa yılan balığı (***Anguilla anguilla ***L., 1758)'nda bazı hematolojik parametrelerin karşılaştırmalı çalışması.*** Araştırma, 2004 yılının yaz döneminde Ceyhan nehri'nin tarımsal, sanayi, mezbaha ve evsel atıklarının deşarj olduğu bölge (Büyükmangıt köyü) ile aynı nehir üzerinde kurulu olan Aslantaş barajı kret altı bölgesi (Osmaniye)'nde yapılmıştır. İstasyonlardaki su kalitesinin olası farklılıklarının hematolojik parametreler üzerine karşılaştırmalı etkilerini ortaya koymak için suyun sıcaklık, kimyasal oksijen ihtiyacı, pH, nitrat nitrojeni (NO_3-N), nitrit nitrojeni (NO_2-N), amonyak nitrojeni (NH_3-N), çözülebilir reaktif fosfor düzeyleri belirlenmiştir. Her bir istasyondan aylık 40'ar balık yakalanmış ve balıklarda hematolojik parametrelerden eritrosit, lökosit miktarları, hemoglobin, lökosit hücre (lenfosit, monosit, nötrofil, ösinofil) oranları ve bu hücrelerin büyüklükleri karşılaştırılmıştır. Su kalite parametreleri açısından Ceyhan nehri'nin kirli olduğu tespit edilmiştir. Ayrıca, bu istasyondan yakalanan balıkların %75'inin hava kesesi paraziti (*Anguillicola crassus*) ile infeste olduğu bulunmuştur. Araştırmada, lökosit miktarı ile nötrofil hücre oranlarının belirtilen stres faktörlerinden dolayı arttığı bildirilmiştir ($P<0,05$).

Anahtar Kelimeler: *Anguilla anguilla*, hematolojik parametreler, su kalite parametreleri, *Anguillicola crassus,* Ceyhan Nehri.

Abstract: This study was carried out in a agricultural, industrial, domestic and slaughterhouse discharging region (around Büyükmangıt Village) of Ceyhan River and just under the dam's crest of Aslantaş (Osmaniye) founded on the same river in the summer season of 2004. Water temperature, chemical oxygen demand (COD), pH, nitrate-nitrogen (NO_3-N), nitrite-nitrogen (NO_2-N), ammonia nitrogen (NH_3-N), and soluble reactive phosphorus (SRP) values in these two stations were determined to the comparative effects of possible differences of water quality on haematological parameters. Forty fish were caught monthly from each station. Erythrocyte, leucocyte, haemoglobine (Hb), leucocyte cell (lymphocyte, monocyte, neutrophil, eosinophil) proportions and sizes of these cells of the fish were compared for two stations. As regard to water quality parameters, Station II was found in contaminated. Moreover, 75% of fish from Station II were found to be infested by swim bladder parasite *Anguillicola crassus*. In the study, leucocyte values and neutrophil proportion were found increased by means of environmental stressors ($P<0.05$).

Key Words: *Anguilla anguilla*, haematological parameters, water quality parameters, *Anguillicola crassus,* Ceyhan river.

Introduction

Fishes may be confronted with stress factors such as varied water qualities, pollution, malnutrition and disease. Fishes can adapt themselves to bad environmental conditions by changing their physiological activities. Qualitative and quantitative variations in haematological parameters including the red blood cell (RBC) and white blood cell (WBC) numbers, cell proportions of leukocyte, the amount of haemoglobin (Hb), and the size of RBC and WBC are the most significant findings as regards diagnosis.

There are many settlements through the Ceyhan River. Ceyhan is the most important town in the study area and one of the important industrial centers for textiles, maize drying, food and beverage, agricultural machinery and oil processing, and cotton-processing sectors (Anonymous, 2000). Baku-Ceyhan Crude Oil Pipeline will also add to the strategic and industrial significance of the Ceyhan area.

This region has also high agricultural potential and the river has been exposed agricultural activities densely in almost four seasons. So, it is thought to be affected from the pollution. In fact, Yılmazer and Yaman (1999) stated that river water could be classified as contaminated with iron (Fe), aluminium (Al), and nickel (Ni), and excessively contaminated with lead (Pb), and cadmium (Cd) elements.

Because of its being under the effect of Mediterranean Climate, summer season in this area has high temperature. As water temperature increases, the rate of chemical reactions and decomposition of organic matter generally increase. Being of pollution sources and additionally, increasing of water temperature are very important problems for aquatic organisms.

It is not known how European eel (*Anguilla anguilla)* is affected by pollution in this region (Anonymous, 2000)).

The aims of the study were to determine the levels of water pollution of the Ceyhan River and to discuss to what

extent the health of European eel of the river had affected from the pollution using haematological parameters. For this purpose, certain water quality parameters of the river and some haematological parameters of this fish caught from the river were analyzed.

Material and Methods

Present study was carried out in summer season because of peaking of the pollution in this season in Ceyhan river (Yılmazer and Yaman 1999).

In the study, water and fish samples were obtained monthly from two stations in Ceyhan River (June, July, August). The stations were chosen considering the agricultural and industrial activities: The first station was just under the dam's crest of Aslantas founded on Ceyhan River, and it was thought to be less affected from the pollution. The second station was in discharging area and thought to be highly polluted (Figure 1).

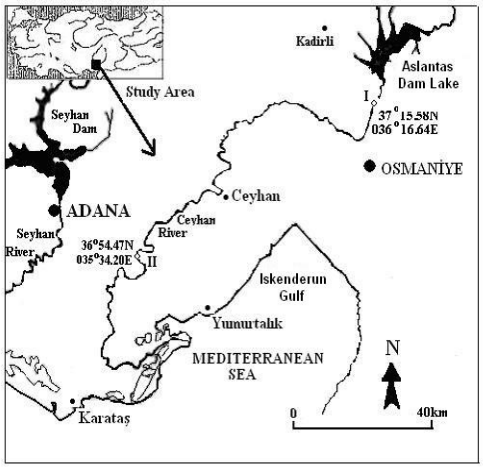

Figure 1. Stations in Ceyhan River, Adana- Turkey.

When fish was sampled, the water samples were collected in triplicate in one liter polyethylene bottles pre-washed with dilute hydrochloric acid and rinsed three to four times with the water sample before filling it to capacity and then labeled accordingly. The bottles were stored under ice.

Temperature and pH of water samples were measured in the field using a digital pH meter (WTW mark). For water quality analyses, the nitrite-nitrogen (NO_2-N) was measured by the colorometric method using sulfanilamide, the nitrate-nitrogen (NO_3-N) by the cadmium reduction method, the ammonia nitrogen (NH_3-N) by the phenate method, the soluble reactive phosphorus (SRP) by the ascorbic acid method, and chemical oxygen demand (COD) by the titrimetric method. Results of water quality parameters for pollution were evaluated according to (APHA 1998).

Forty individuals were caught in each month from each station using same fishing line and extension net. Fish samples were put into transport tank (270 L) fulled with water of river in which fish were sampled. Fish and water samples were transported to Fish Disease Laboratory and Water Quality and Chemistry Laboratory of Fisheries Faculty, Cukurova University, on the same day.

During transport, water of the tank was oxygenated. When brought to the laboratory, fish was measured. After total length and body weight were measured, fish were investigated for ecto and endoparasites (Bauer 1987, Moravec 1994, Qang 1998, Singhal 1984, Wootten, 1989).

Blood samples from caudal vein of each fish were taken by means of an injector and put into the tubes with EDTA. Red blood cells were counted by using Natt-Herrick solution, and the amount of white blood cells was measured by using Thoma micro-slide (Blaxhall 1972, Konuk 1981). Syanmethaemoglobin method was used in Hb (haemoglobin) determination (Blaxhall 1973, Tanyer 1985). Peripheric blood smears (PBS) were dyed with the mixture of May-Grünwald and Giemsa. Percentage of leukocyte cells was determined using these preparations (Kocabatmaz and Ekingen 1984, Fujimaki and Isoda 1990). The diameters of RBC, lymphocyte, monocyte, neutrophil and eosinophil in dyed preparations were measured with ocular micrometer.

The result obtained monthly (June, July, August) in the summer were presented as only one mean for this season. Statistical analyses were carried out using SPSS 10.0 packet program (Anonymous 1999). T-test (at 0.05 significance level) was applied for haematologic parameters of the fish.

Results

All water quality parameters in Station I were found lower than those in the station II in this study (Table 1). Station II was determined as polluted according to WHO (1996).

Average values of the total length and body weight of the fish were 57.60±11.59cm and 667.90±19.40g for Station I, and 59.48±3.10cm and 401.70±36.80g for Station II, respectively

As a result of examination of the fish, any health problem was observed except from endoparasite. As a consequence of parasitological analysis, *Anguillicola crassus* (Nematode) was observed in 90 out of 120 specimens (75%) of Station II, whereas it was not found in the fish of Station I. Averages values of water quality parameters are given in Table 1. The results of haematological analyses were presented in detail in Table 2 and 3.

According to the haematologic results, only WBC and neutrophil values of fish caught from Stations I and II were statistically significant ($P<0.05$). No significant difference ($P>0.05$) was found between fish caught from Stations I and II, with respect to the quantity or size or the structure of erythrocyte cells obtained from PBS prepared.

Table 1. Water quality parameters in Ceyhan River.

	Parameters						
Stations	Temperature (°C)	pH	NO_3-N (mgL^{-1})	NO_2-N (mgL^{-1})	NH_3-N (mgL^{-1})	SRP (mgL^{-1})	COD (mgL^{-1})
I	21.06±7.4*	8.10±0.08	0.55±0.01*	0.007±0.002*	0.25±0.20*	0.001±0.001*	8.81±3.16*
II	26.8 ±7.5	7.21±0.02	1.26±0.01	0.04±0.01	0.45±0.02	0.03±0.01	37.75±6.41

*X±SD: Mean Value ± Standart Deviation; *: p<0.05 significant level*

Table 2. RBC, WBC and Hb Values of European eel in Ceyhan River. RBC: Erythrocyte Cells, WBC: Leukocyte Cells, Hb: Hemoglobin.

Stations	RBC ($10^6/mm^3$)	WBC ($10^3/mm^3$)	Hb (g/dl)
I	1.30±367.52 ((1.090-1.96)	2.38±9.03* (1.10-3.60)	10.14±0.96 (9.40-11.8)
II	1.28±44.08 (0.54-1.93)	3.75±1.29 (2.05-5.50)	9.86±1.12 (8.0-11.5)

*X±SD: Mean Value ± Standart Deviation; (minimum and maximum values); *: p<0.05 significant level*

Table 3. Leukocyte proportions and sizes of blood cells of European eel in Ceyhan River. LC: Leukocyte Cells. LQ, MQ, NQ, EOQ: the numbers of lymphocyte, monosite, neutrophil, eosinophil, cells, respectively LS, MS, NS, EOS, ES: the sizes of lymphocyte, monocyte, neutrophil, eosinophil, erythrocyte cells, respectively

LC	Lymphocyte		Monocyte		Neutrophil		Eosinophil		Erythroc.
Stations	LQ (%)	LS (µm)	MQ (%)	MS (µm)	NQ (%)	NS (µm)	EOQ (%)	EOS (µm)	ES (µm)
I	50.20±19.0 (26-76)	13.14±1.7 (9.5-15)	46.0±18.8 (22-70)	9.50±1.2 (7.5-11)	3.40±1.3* (2-6)	8.28±1.1 (7-10)	2.0±0.3 (2-2)	9.28±2.4 (7-13)	13.5±0.4 (13-14)
II	45.40±20.3 (26-80)	11.71±1.7 (10-14)	45.0±15.4 (20-68)	8.85±1.7 (7-11)	10.85±8.8 (2-26)	10.80±3.1 (7-15)	2.50±0.9 (2-2)	8.50±2.1 (7-10)	14.07±1.0 (13-16)

*X±SD: Mean Value ± Standart Deviation; (minimum and maximum values); *: p<0.05 significant level*

Discussion

Ceyhan river exposes to intensively agricultural wastes during nearly four seasons of the year. In a previous study, iron Fe), aluminium (Al), nicel (Ni), lead (Pb) and cadmium (Cd) were found in this river water (Yılmazer and Yaman 1999).

COD results of our study was consistency with WHO (1996) which reported the concentrations of COD observed in surface waters range from 20 mg/l O_2 or less in unpolluted waters to greater than 200 mg/l O_2 in waters receiving effluents.

In the usual case, freshwater is below 0.001 mg/l for nitrite. High concentrations of nitrite are often associated with unsatisfactory microbiological water quality and industrial effluents (WHO 1994).

In rural and suburban areas, the use of inorganic nitrate fertilizers can be a significant sources for nitrate. When influenced by human activities, surface waters can have nitrate concentrations up to 5 mgl^{-1} NO_3-N, but often less than 1 mgl^{-1} NO_3-N in unpolluted waters. Levels in excess of 0.2 mg/l nitrate indicate possible eutrophic conditions in freshwater (WHO 1994). Because nitrate values of this river excess 0.2 mg/l, the river can be in eutrophic condition.

Total ammonia concentrations measured in surface waters are typically less than 0.2 mg/l N but may reach 2-3 mg/l N. Higher concentrations can be an indication of organic pollution such as from domestic sewage, industrial waste and fertilizer run-off (WHO 1994). In this study, station I has 0.25±0.20 mg/l NH_3-N, whereas station II has 0.45±0.02 mg/l NH_3-N.

In a study that was conducted at two stations at downstream of Ceyhan River, nitrogen compounds were found to be higher than the limits defined for 1st Class quality.

Amonia and nitrate nitrogen values in this study were harmonius with the results of Yılmazer and Yaman (1999). Ammonia and nitrate nitrogen values of our study as well as their results may be the indicators of the negative impacts of agricultural and industrial activities on the water quality of Ceyhan River.

In most natural surface waters, phosphorus ranges from 0.005 to 0.020 mg/l PO_4-P (WHO 1994). In this study, the average of Station II exceeded that level. This concentrations can point out input of domestic and industrial waste-waters and fertilizers. Water quality parameters of Ceyhan river except Station I showed that river is affected by agricultural and industrial activities and discharging from domestic sources.

Due to lack of biological studies on European eel in the region, it is impossible to make a comparative analysis. Therefore, the comparison was carried out with the results of the studies performed in different regions.

In a study performed over carp caught from different regions of Seyhan River whose pollution had been proven in this concept, it was reported that any difference was observed in erythrocyte, haemoglobin and haematocrit values, and that there was a significant increase in WBC quantity and leukocyte cell proportions (lymphocyte, monocyte, and neutrophil percentages) (Şahan and Cengizler 2002). As for our study, WBC quantity increased from 2.38±9.03/mm^3 in the fish of Station I to 3.75±1.29/mm^3 in the fish of Station II (polluted) of Ceyhan river, and neutrophil cell proportion increased from 3.40±1.3% to 10.85±8.8%, respectively, which

was an important increase.

In fish, any infestation with any organism activates the cellular and humoral immune system. This is followed by changes in circulating antibodies and percentages and absolute number of the different WBC (Boon *et al*. 1990).

Quality and quantity of leucocyte cells which are haematologic parameters are generally used to determined of immune reactions and diseases (Ekingen 1988, Çağırgan 1990)

In the present study, the increases in WBC and neutrophil quantities in the samples collected from Station II were accepted as a response of cellular immune system to pollution. It can be concluded from either this study or the studies of Uluköy and Timur (1993), Palikova and Navratil (2001) Şahan and Cengizler (2002) that immune system of fish creates similar responses to unfavorable conditions.

Genç et al. (2005) reported that they had observed the presence of *Anguilla crassus,* an air bladder nematode, in 82.86% of 64 European eels collected from Ceyhan River in summer season, and 72.41% of 56 individuals in winter season. The infestation finding of the proportion of 75% in our study shows similarity with the previous values determined for the fish of the same region by Genç *et al*. (2005). Boon et al. (1989) determined that *A. crassus* was a blood sucker parasite, and its intestine was completely filled with host's erythrocyte. Researchers stressed that *A. crassus* could swallow erythrocyte cells thanks to their flexible membrane structure and could produce a secretion to change lipid structure on cell wall. They also stressed that erythrocyte amount of fish infected with parasite was significantly lower in comparison to those in non-infected ones. The results belonging to erythrocyte of fishes from polluted and unpolluted stations of Ceyhan River were not significant statistically ($P>0.05$). It was reported that there was no significant difference between the fish infected with the parasite and non-infected ones in terms of Hct level, but it was reported that after 7 weeks following infection, Hct and plasma protein levels decreased, WBC quantities reached the highest level in fishes infected with parasite, and that there was an adverse relation between the percentages of lymphocyte and granulocyte (Boon *et al*. 1989).

It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases. In this study, there is a close similarity between the researchers' findings and the increase occurred in leukocyte cells of fish infected with the parasite. A decrease in the percentage of lymphocyte and an increase in the percentage of granulocyte (neutrophil and eosinophil) were seen in the fishes infected. The adverse relation among the cell groups mentioned above gives countenance to the findings of Boon *et. al*. (1990)

Van Der Heijden et al. (1996) determined an increase in the numbers of lymphocyte and granulocyte cells of fish infected with *A. crassus,* and they claimed that a cellular response to this parasite from specific antibodies in eels infected could be produced in time, and certain fish were less affected thanks to their resistance mechanism derived from genetic variation as respect physiology. In our study, any difference in RBC and Hb values was observed in terms of both conditions ($P>0.05$). That the RBC quantity was not affected by *A. crassus,* as mentioned by Van Der Heijden et al. (1996), could be derived from a resistance related to the antibodies developed against parasite in time.

As a result of analysis performed over size and morphology of blood cells, no difference was found between unpolluted and polluted water conditions, in other words, between infected and non-infected fish. This result also indicated that there was no structural defect in morphological structure of blood cells. Despite the existence of pollution, no significant alteration was observed in RBC quantity ($P>0.05$). However, significant increases were seen in WBC quantities and neutrophil cell percentage ($P<0.05$). It is thought that this increase was a respond to deterioration of fish health and being stimulated of the cellular defense mechanism which had a significant role in immune system.

It was reported that length and weight discrepancies of various fish species had no significant effect on haematological parameters (Houston and Wilde 1972, Van Vuren and Hattingh 1978). For this reason, it was presumed in our study that length and weight discrepancies of European eels obtained from unpolluted and polluted water conditions had no effect on haematological parameters measured. Because of the lacking of a similar study performed on European eels in the region, Turkey, this study is quite important not only to provide some meaningful information for the health conditions of this particular species but also to enable us to make some inferences about some other fishes living in the same region as regards forming groundwork for prospective studies.

References

Anonymous, 1999. Computer progm.MS.for Windows, version 10.0 SPSS Inc., USA.

Anonymous, 2000. .Aslantas Dam and related aspects of the Ceyhan River Basin, Turkey. A WCD case study prepared as an input to the World Commission on Dams. Agrin Co. Ltd. Cape Town. www.dams.org.

APHA (American Public Health Association), 1998. Standard methods for the examination of water and wastewater. 20th Edition. Washington.

Bauer, O.N. 1987. Key to the parasites of freshwater fishes of the fauna USSR, Part 3, Nauka, Leningrad, 583 p.

Blaxhall, P.C. 1972. The haematological assessment of the health of freshwater fish. J. of Fish Biology, 4: 593-604.

Blaxhall, P.C., K.W. Daisley, 1973. Routine haematological methods for use with fish blood. J. of Fish Biology,; 5: 771-882.

Boon, J.H., C.J.A. Lokin, R. Ceusters, F. Ollevier, 1989. Some properties of the blood of European eel (*Anguilla anguilla*) and the possible relationship with *Anguillicola crassus* infestations. Aquaculture, 76: 203-208.

Boon, J.H., V.M.H. Cannaerts, H. Augustijn, M.A.M. Machiels, D. De Charleroy, F. Ollevier, 1990. The effect of different infection levels with infective larvae of *Anguillicola crassus* on haematological parameters of European eel (*Anguilla anguilla*). Aquaculture, 87: 243-253.

Çağırgan, H. 1990. Immune system in teleost and its significance on diagnosis (in Turkish). Uni.of Aegean, J.of Fısheries and Aquatic Sciences 7, (25, 26, 27, 28), 57-68.

Ekingen, G. 1988. The significance on science and uses of fish haematology (in Turkish). Univ. of Fırat, Faculty of Veterinary Publ. Elazığ. (4), 1-5.

Fujımakı, Y., M. Isoda, 1990. Fine-Structural of Leucocytes in the Goldfish, *Carassius auratus*. J. of Fish Biol., England, (36), p. 821-831.

Genç, E., A. Şahan, T. Altun, İ. Cengizler, E. Nevsat, 2005. Occurrence of the swimbladder parasite *Anguillicola crassus* (Nematoda, Dracunculoidea) in European eels (*Anguilla anguilla*) in Ceyhan River, Turkey. Turk. J. Vet. Anim. Sci., 29: 661-663.

Houston, A.H., M.A. Wilde, 1972. Some observations upon relationship of microhaematocrit values to haemoglobin concentrations and erythrocyte numbers in the carp (*Cyprinus carpio* L.) and brook trout *Salvelinus fontinalis* (Mitchill). J. of Fish Biol. 4: 109-115.

Kocabatmaz, M., G. Ekingen, 1984. Standardization of haematological methods and taking blood from various fish species, (in Turkish). J. of Nature Sci., 2: 149-159.

Konuk, T. 1981. Practical Physiology. 1st Publication of Veterinary Faculty, Ankara Univ. Ankara.; 181 p.

Moravec, F., 1994. Parasitic nematodes of freshwater fishes of Europe. Prag., 473p.

Palikova, M., S. Navratil, 2001. Occurrence of *Anguillicola crassus* in the water reservoir Korycany (Czech Republic) and its influence on the health condition and haematological indices of eels. Acta Vet. Brno. 70: 443-449.

Qang,T.B., 1998. Parasitic fauna of the freshwater fish of the Cuulong River Delta (Lower Mekong River Delta) and methods for prevention and treatment. The Aquatic Animal Health Research Institute. Newsletter article, 7: 1-13.

Singhal, R.N., S. Jeet, R.W. Davies, 1984. Ectoparasites of the fresh water food fishes of Hayrana. Proc. Indian. Acad. Sci. (Anim. Science), 93: 663-669.

Şahan, A., İ. Cengizler, 2002. Determination of some heamatological parameters in spotted barb (*Capoeta barroisi,* 1894) and roach (*Rutilus rutilus*, 1758) Living in Seyhan River (Adana City Region), (in Turkish). Turk. J. Vet. Anim. Sci., 26: 849-858.

Tanyer, G. 1985. Haematology and laboratory. Textbook. Ayyıldız Publication. (in Turkish) Ankara. 441p.

Uluköy, G., M. Timur, 1993. A study on observation of haematological and histopathological changes occurred from some pesticides in different concentrations in pikeperch (*Stizostedion lucioperca* L. 1758), ((in Turkish). J. of Fisheries and Aquatic Science, 10: 35-54.

Van Der Heijden, M.H.T., G.H.R. Booms, M.W.T. Tanck, J.H.W.M. Rombout, J.H. Boon, 1996. Influence of flumequine on invivo mitogen responses of European eel (*Anguilla anguilla* L., 1758) lymphoid cells. Veterinary Immunology and Immunopathology, 47: 143-152.

Vuren, J.H., J. Hattingh, 1978. A seasonal study of haematology of wild freshwater fish. J. of Fish Biol., 13: 305-313.

Who. 1994. Water quality assessments: a guide to the use of biota, sediments and water in environmental monitoring. 2nd ed., Spon, 651p.

Wootten, R. 1989. The parasitology of teleosts. In: Roberts, R.J., Eds. Fish Pathology. 2nd Ed., Bailliere Tindall, London.; 242-287.

Yılmazer, D., S. Yaman, 1999. Heavy Metal Pollution and Chemical Profile of Ceyhan river (Adana-Turkey), (in Turkish). Tr. J. of Engineering and Environmental Science, 23:59-61.