

Ecophysiological Effects of Ichthyotoxic (*Verbascum sp.*) on some Local Fishes in Sulaimani City- Iraq

Raza H. Husein Rezan O. Rasheed
Sulaimani University, College of Science-dept. Biology.

Abstracts

In the present study 2kg from the Arial parts (shoots, leaves, flowers) of the plant mullein (*Verbascum sp.*) were collected from Sharbazher province in Sulaimani city, during the summer season ,the specimen was deposited in herbarium of biology dept. Sulaimani university. Each parts of plant were grinded separately, given to the *Cyprinus carpio* fish in different times and groups with different dose according to concentrations. Low (10gm), moderate (40gm), and high (90gm) concentrations were used. Ecological factors were conceders by the assessment of water temperature, pH, turbidity, electrical conductivity, Total dissolved solids, and dissolved oxygen. Physiological study includes some haematological parameters, and histopathological effects of plant on liver, brain and heart sections. There is a significant difference between some haematological parameters and concentrations.

Key words: *Verbascum sp.* poisoning plants, ichthyotoxic, fish physiology

الخلاصة

carpio Cyprinus على اسماك الكارب المحلية *Verbascum sp* دراسة تاثير النبات تمت وذلك عن طريق جمع النماذج النباتية في منطقة شاربازير الواقعة ضمن محافظة السليمانية لاجل معرفة التاثير السمي للنبات كما درست بعض العوامل البيئية للمياه كدرجة الحرارة الماء ,الاس الهيدروجيني, التوصيلية الكهربائية, كمية الاملاح الذائبة , العكورة , اضافة الى كمية الاوكسجين المذاب من جهة و من جهة اخرى تمت دراسة بعض العوامل الفسيولوجية مثل معايير الدم و كذلك التاثيرات النسيجية المرضية للكبد,الدماغ,ومقاطع القلب لاسماك الكارب بعدما اضيفت التراكيز المختلفة من النبات (10%, 40%, و 90%).

اظهرت النتائج وجود فروقات معنوية لبعض معايير الدموية مع التراكيز المختلفة من النبات .

Introduction

Since ancient times *Verbascum sp.* has been used as fish poison, and recorded by Aristotle as a fish poison (Gross and Werner 1978). Until now some native preserved this culture and using poisonous plants also called ichthyotoxins to catch fish (Kritzon, 2003)

Exposure of aquatic organisms to some organic compound like verbascum plant, water soluble- and water accommodated fractions of plant have been shown to impact on various aspects of fish physiology and sometimes leading to large scale mortality (Barron *et al.*, 2003; Couillard *et al.*, 2005). Several workers have revealed histopathological changes in various organs of fish (ovaries, air sac, brain, gill and liver) exposed to plant toxicity (Stott *et al.*, 1981; Akaishia *et al.*, 2004).

Verbascum species contain a wide range of compounds, such as glycosides (Klimek, 1996), alkaloids (Youhnovski *et al.*, 1999) and saponins (Hartleb & Seifert, 1994). In addition, species of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkaloids (Koblikova *et al.*, 1983; Seifert *et al.* 1982).

Verbascum species are poisonous because of the saponins contained. People use these poisonous seeds for hunting fish. *Verbascum* species are called 'fishplant' in the nothern Anatolia because of that property (Zeybek, 1985; Baytop, 1999). This study was undertaken to assess the haematological and histopathological responses of some

organ in fish by using low, moderate, and high concentrations of verbascum plant under laboratory conditions.

Materials and methods

Verbascum species were collected from Sharbazher provide in Sulaimani city and identified by prof. Dr. **Abdul-ussein Alkhayat** and the specimen was deposited in herbarium of biology dept. Sulaimani university. Each Aerial parts of verbascum plant (leaves, flowers, and seeds) was grind to fine powder. Ten (10), forty (40), ninety (90) grams of the powder were measured.

Aquaria treatments:

In the present study bath treatment was used. Aquaria treatments are a popular method of administering of crude plant to achieve the desired result as compared to oral treatments. In many cases, even a large amount of plant grind in the water does not guarantee that enough of it will get into the fish to be an effective treatment. At the same time, excessive amounts in the bath treatments are remain in the water.

Fish housing

Sixteen (16) fish *Cyprinus carpio* were used in the study. Average weighing about 2-2.5 Kg. Fish was housed in the animal house in four rectangular aquaria in Ecology lab. Biology Dept. The fish were reared under standard laboratory conditions.

Ecological study:

Some ecological parameters were studied including each of water temperature in C°, pH, Electrical Conductivity (EC) ($\mu\text{S cm}^{-1}$), Total dissolved solid (TDS) in mg l^{-1} , Turbidity (NTU), and Dissolved Oxygen (DO) in (mg l^{-1}). a multimeter of model TPS90FL-T. Field Lab Analyzer, was used according to (A.P.H.A.,2005).

Experimental design

This experiment was designed to Eco-physiological study of mullein plant on haematological and histopathological parameters of some organ in fish as the following:

Group1: Control (first aquarium) 4 fish

This group was kept in the water without any treatments

Group2: Verbascum crude (10g) (second aquarium) 4 fish

This group was given 10gm of verbascum grind in the water

Group3: Verbascum crude (40g) (third aquarium) 4 fish

This group was given 40gm of verbascum grind in the water

Group4: Verbascum crude (90g) (fourth aquarium) 4 fish

This group was given 90gm of verbascum grind in the water

The samples collection

At the end of the experiment, blood samples were collected from the fish by cardiac puncture and analysed for Haemoglobin (Hb), Red Blood Cells (RBC) count, Thrombocytes and one parameters of red blood cell indices (MCH). Liver, Brain, air sac, and the heart biopsy were obtained from exposed fish and control, preserved in 10% formalin and processed for histological examination (using standard histological techniques. Sections of organs were cut at $5\mu\text{m}$ and stained with haematoxylin and eosin. Permanent sections were read under light microscope.

Results and discussions

1- Ecological studies:

During this study, some ecological parameters were investigated, as shown in table (1), results revealed that *Verbascum sp.* was not effect on the water temperature in different concentrations except in 90 gm, this may be due to the high concentration was used which act as a cooling agent of the water as well as it act as an insulating

material between water temperature and air temperature of the lab. The pH of the water samples was not affected in first two concentrations of (10% & 40%) with recorded values of (7.1 & 7.0) while at concentration of 90gm the pH was decreased to about 6.93, this value indicated that *Verbascum sp.* was dissociated in addition of decreasing DO which indirectly increase CO₂ (Odum, 1971). On the other hand, each of Electrical conductivity and TDS were increased, because of the chemical composition of *Verbascum sp.* (Couillard *et al.*, 2005). Increase in the Turbidity of water samples after addition of this plant, when in each concentration of (10gm, 40gm, & 90gm) added to the water, the turbidity value was (5, 5, & 6 NTU), this may be due to the particles of grinded parts, when suspended in the water column, and decreased visibility. (Shaw, *et al.*, 2007). DO is one of the most important factor for all living organisms especially fish survive (Bartram, and Balance, 1996). As shown in table (1), DO of the water samples were decreased by increasing concentration of *Verbascum sp.* When the DO concentration of the water sample was (6.43 mg l⁻¹), but it was decreased to about (3.5, 4.5, & 4.3 mg l⁻¹) for the concentrations of (10gm, 40gm, & 90gm) respectively, these results revealed that *Verbascum sp.* Have significant effects on the water quality in general and DO concentration in special because the consumption of DO by microorganism in decomposition processes.

2- Histopathological study:

From 100 tissues slide the following slides have been selected to show the destructive and toxicity effects of verbascum plant on fish. The brain sections from the control were normal without infiltration of glial cell and prevascularization cuffing (plate A). Microscopic finding of brain sections after given 40g and 90g of verbascum were causes of mild injury such as; congestion of blood vessels, infiltration of glial cells, prevascularization cuffing and some times demyelination obvious in some fields (plate B & C). Plate D, E & F show the effects of verbascum plant on heart tissue, in some finding of 40gm and 90gm of verbascum congestion of blood vessel and haemorrhage were present in comparison to control. Plate G show microscopic finding of liver section after treated fish with 90gm of verbascum, in liver it is so clear the toxicity of verbascum on tissue and that can be observed vaculation of hepatocyte oedema (swilling), inflammatory infiltration and haemorrhage. Degrees of the pathological changes in the each tissue were directly concentration-dependent. The two primary chemicals that occur in most plants and poisonous to fish are saponin and rotenone, these normally effect on most of organs present in the fish body especially destructive digestive, cardiovascular, respiratory, nervous system, Even the effects of the poison are powerful they are not usually fatal may be return to their pre-toxic condition, for this reason this plant use to catch fish in many places in the world (Campbell, 1991).

3- Haematological parameters:

One of the chemical components of *verbascum sp.* plant is saponin, saponin normally breakdown in the digestive system and enter the blood stream to be toxic, but also fish take it directly through their gills (Kritzon, 2003). As shown in figure 1 and 2 haemoglobin concentration and red blood cells count of the fish did not changed significantly ($p \geq 0.05$) by gradient concentration of verbascum plant when compared to control fish. The concentration of erythrocytes in the blood can be expressed as haemoglobin concentration or as the number of red cells per volume blood. In the figures 3 and 4 some values of MCH and thrombocytes vary by concentrations of mullein plant. The value of MCH in 10gm and 40gm concentration of plant did not changed significantly while 90gm increased significantly, although according to value of thrombocytes in 10gm concentration of plant did not changed

significantly ($p \geq 0.05$) while 90gm and 40gm increased significantly ($p \leq 0.05$) in comparison to control group. Shoot part of verbascum species are used for fishing because of their saponin contents which are toxic to fish (Bytop, 1999)

Haematological parameters are very sensitive to stress like oil crude, toxicant plant and other environmental changes and some studies performed on this issue. Omoregie, (1998) and Gabriele (2007) reported in *C. gariepinus* and *Oreochromis niloticus* fish decrease in the values of Hb, RBC count, HCT, MCV with increase in the concentration of the refined crude oil, similar study recorded a decrease in the values of all the haematological parameters but a significant increase in the Hb value of the catfish, *H. fossilis* exposed to crude oil (Prasad *et al.* 1987). The decrease in the values of these parameters could be attributed to haemolysis resulting in the mechanism for diluting the concentration of the pollutant in the circulatory system (Smith *et al.*, 1979). Erythropania recorded in the exposed fish may also be accounted for by swelling of the erythrocytes (Annune and Ahuma, 1998), damages to haematopoietic tissues in the kidneys and aggregation of cells at the gills thereby causing a decrease in the number of circulating cells of stressed fish (Singh and Singh, 1982).

Changes in the values of haematological parameters in these studies as well as ours were concentration- dependent. This may be due to the level as well as duration of exposure to the toxicant. In table 1 the period of fish mortality with different concentration and duration was obvious, the mortality was increased by increasing concentration of grind verbascum plant.

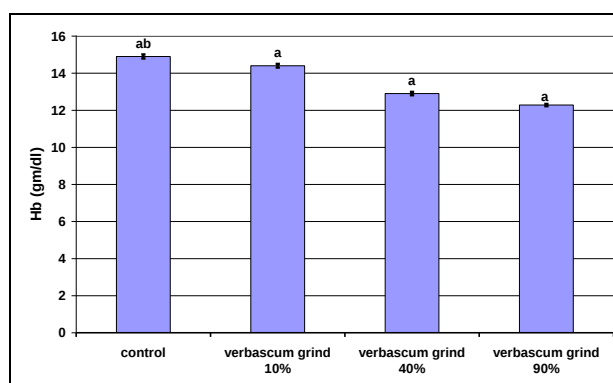


Figure 1: Effects of different concentration of grind verbascum plant on haemoglobin concentration of *Cyprinus carpio*.

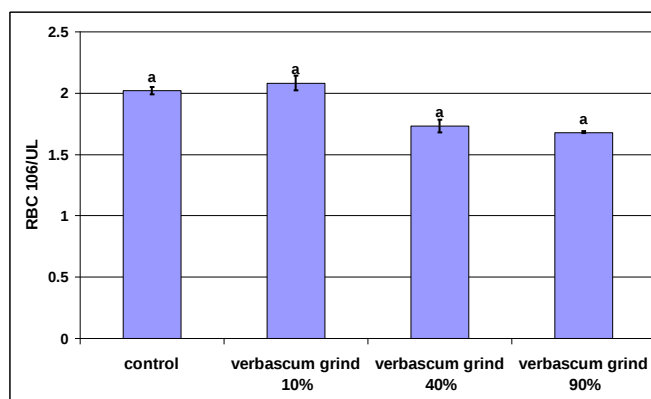


Figure 1: Effects of different concentration of crude verbascum plants on RBC count of *Cyprinus carpio*

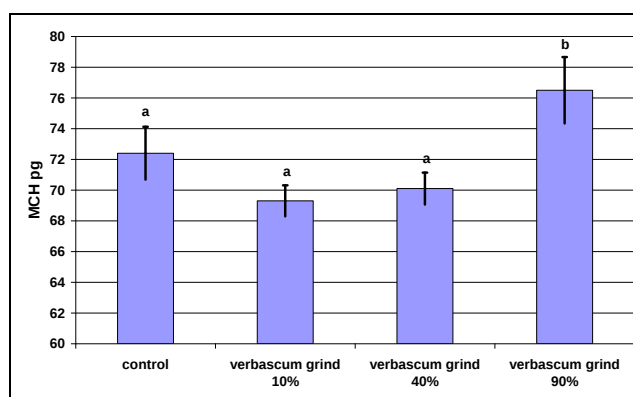


Figure 1: Effects of different concentration of crude *verbascum* plants on MCH of *Cyprinus carpio*

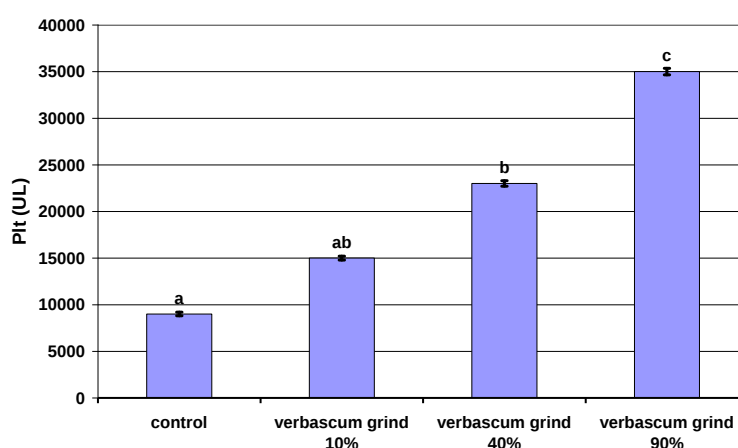


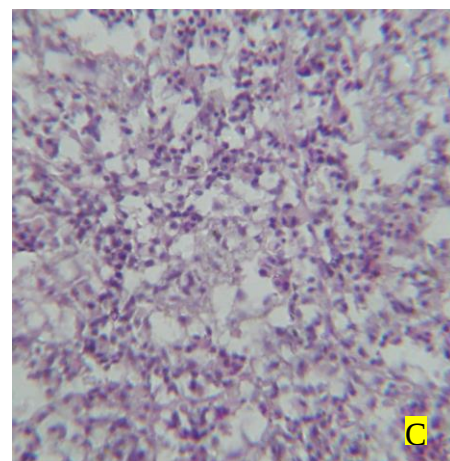
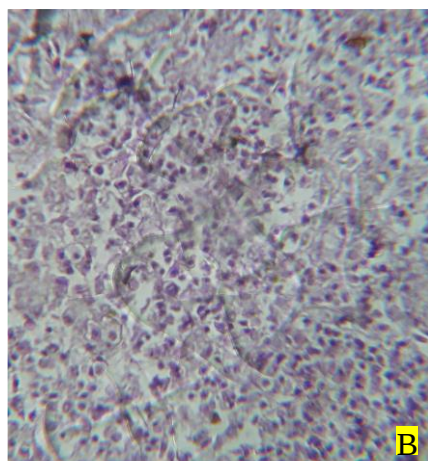
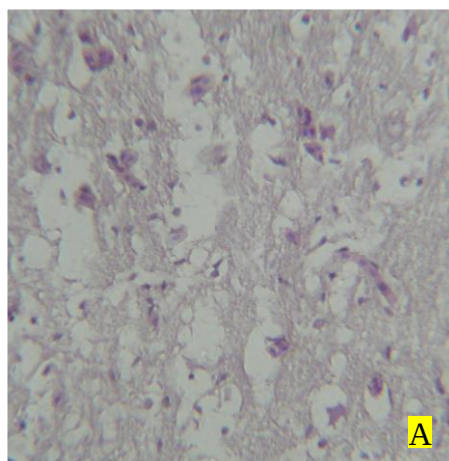
Figure 1: Effects of different concentration of crude *verbascum* plants on platelet count of *Cyprinus carpio*

Table (1): Shows some ecological parameters for different concentrations.

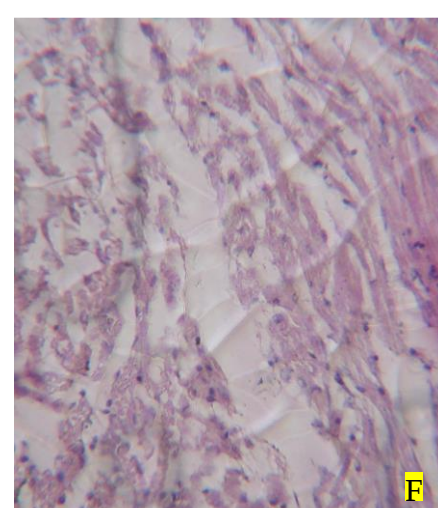
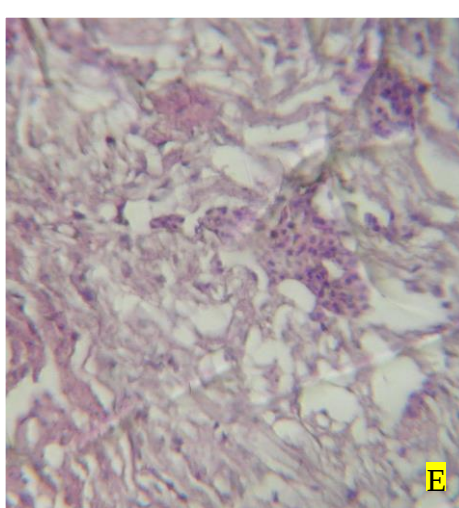
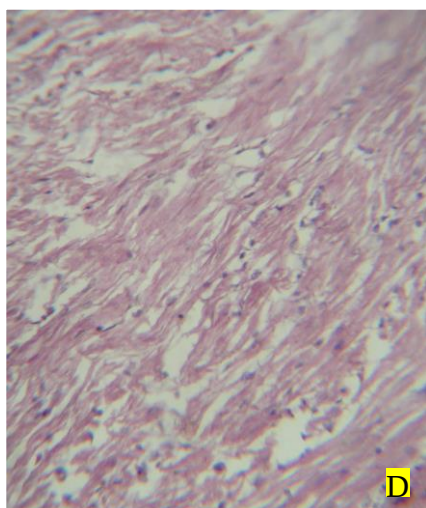
Ecological parameters	water samples before treatment	water samples with Conc.(%10)	water samples with Conc.(%40)	water samples with Conc.(%90)
Water temperature (C°)	30	30	31	23.3
pH	7.11	7	7.22	6.93
EC ($\mu\text{S cm}^{-1}$)	567	569	580	635
TDS(mg l^{-1})	286	287	292	324
Turbidity (NTU)	2	5	5	6
DO (mg l^{-1})	6.43	3.5	4.5	4.3

Table (2): Shows the death time in (hours) of *Cyprinus carpio* for different concentrations of *Verbascum sp.*

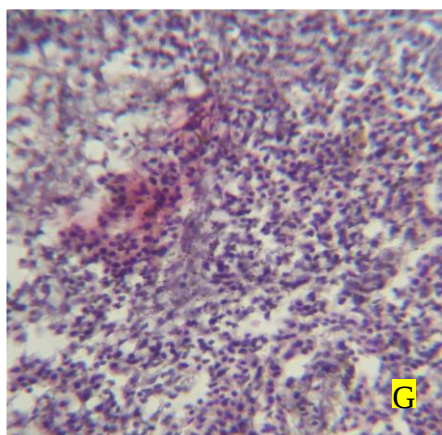
treatments	Period of killing <i>Cyprinus carpio</i>	
	male	Female
control	48 hour	36 hours
10%	12 hours	10 hours
40%	1.30 hours	3 hours
90%	0.30 hours	1 hours



Brain section: (A) Control. (B) Fish given 40gm of *verbascum sp.* crude in the water. (C) Fish given 90gm of *verbascum sp.* crude in the water.



Heart section: (D) Control. (E) Fish given 40gm of *verbascum sp.* crude in the water. (F) fish given 90gm of *verbascum sp.* crude in the water.



Liver section: (G) fish given 90gm of *verbascum sp.* crude in the water.

References

- Akaisha, F.M., FtC De Assis, S.C. Iaakobi, D.R. Eiras Stofella, S.D. St. lean, S.C. Couteany, E.F. Lima, A.L. Wagener, A.L. Scofield, and C.A. Ribeiro, 2004. Morpholoical and neurotoxicological findings in tropical freshwater fish (*Astyanax* sp.) after waterborne and acute exposure to water soluble fraction of crude oil. Arch. Environ. Contam. Toxicol., 46: 244-253
- American Public Health Association A. P. H. A. (2005). Standard Methods for Examination of Water and Waste Water. 21th ed. Washington DC, USA.
- Annune, P.A. and F.T.A. Ahuma, 1998. Changes in mudfish exposed concentrations of copper and lead. Acta drochem. *Hydrobiol* 1.3: 33-36.
- Barron, M.G., M.G. Carls, J.W. Short and S.D. Rice, 2003. Photoenhanced toxicity of aqueous phase and chemically dispersd weathered Alska North Slope crude oil to Pacific hering eggs and and larvae. Environ. Toxicol. Chem., 22: 650-660.
- Banerjee, V., 1. 998. Influence o zinc and mercury on blood parameters of the fish, *Heteropneutes fossilis*. Environ. Ecol., 1. 6: 79-84
- Bartram, J. and Balance, R. (1996). Water quality Monitoring. E and FN Spon, an imprint of Chapman and Hall. London. pp382.
- Baytop T (1999): Theraphy with Medicinal Plants in Turkey (Past and Present). (in Turkish) 2nd Ed. Nobel Tıp Kitabevleri, pp. 334–335.
- Campbell, paul, (1991). “Fish poison” survival skills of native California. Gibbis Smith publishing, Layton Utah. PP.433-434,
- Couillard, C.M., K. lee, B. Legare and T.L. King, (2005). Effect of dispersnt on the composition of the water soluble-accommodated fraction of crude oil and its toxicity to larval marine fish. Environ. Toxicol. Chem., 24: 1.496-1.4504. Haem atol og i cal to sublethal. Aquat. Sci.,
- Elgindi MR, Mabry TJ (2000): Phenyletanoid glycosides from *Verbascum siniaticum*. *Asian J Chem* 12: 127–130.
- Gabriel, U.U.; Amakiri E.U. and Ezeri G.N.O. (2007) Haematology and Gill Pathology of *Clarias Gariepinus* Exposed to Refined Petroleum Oil, Kerosene under Laboratory Conditions; *Journal of Animal and Veterinary Advances* 6 (3): 46 -465
- Gross, K.L. and P.A. Werner. 1978. The biology of Canadian weeds: *Verbascum thapsus* and *V.blatteria*. *Can. J. Plant Science* 58:401-413.
- Hartleb I, and Seifert K (1994): Sangarosaponin-D a triterpenoid saponin from *Verbacsum songaricum*. *Phytochemistry* 35: 1009–1011.
- Kalpoutzakis E, Aligiannis N, Mitakou S, Skaltsounis AL (1999): Verbaspinoside, a new iridoid glycoside from *Verbascum spinosum*. *J Nat Prod* 62: 342–344.
- Klimek B (1996): Hidrooxycinnamoyl ester glycosides and saponins from flowers of *Verbascum phlomoides*. *Phytochemistry* 43: 1281–1284.
- Koblikova Z, Turecek F, Ninova P, Trojanek J, Blaha K (1983): Verbaskine, a macrocyclic spermin alcholoide of a novel type from *Verbascum pseudonobile* Stoj. et Stef. (Scrophulariaceae). *Tet Lett* 24: 4381–4384.
- Kritzon C. (2003) Fshing with poisons. *Bulletin of primitive technology.spring*,#25.
- Odum, E. P. (1971). Fundamentals of Ecology. W. B. Saunders Company. US of America. pp 312
- Omoriegie, E., (1998). Changes in the haematology of Nile tilapia, *Oreochromis niloticus* under toxicological effects of commercial diesel in Nigerian the effects of crude oil. Acta Hydrobiol., 40: 287-292.
- Prasad, M.S., M. Prasad and D. Singh, 987. Some haematological effects of crude oil on fresh water fish, *Heteropneustes fossilis*. Acta drochem. *Hydrobiol.*, 5: 99-204.

- Seifert KH, Jahne and S, Hesse M (1982): Verbascenine, ein macrocyclische spermin alkaloid aus Verbascum. *Helv Chim Acta* 65: 2540–2547
- Shaw, Byron; Mechenich, Christine and Klessig, Lowell. (2007). Understanding lake Data. By intrnet [www. dnr. state. wi. us/org/water/fhp/lakes/under/](http://www.dnr.state.wi.us/org/water/fhp/lakes/under/) - 10k.
- Singh A.and Singh, B. (1982). Effects of copper and zinc sulphate on the blood parameters of *Mystus vittatus*. **Matsya**, 8: 1-6.
- Skaltsounis AL, Tsitsa-Tzardis E, Demetzos C, and Harvala C (1996): Unduloside, a new iridoid glycoside from *Verbascum undulatum*. *J Nat Prod* 59: 673–675.
- Smith, G.L.; Hattingh, J.; and Burger, A. P. (1979) Haematological assemenrt of anaesthesia, MS 222 in natural and neutral forming three freshwater fish species: interspecific differences. *J. Fish Bio.*, 15. 633-643
- Stott, G.G., N. H. McArthur, R. Tarpley, V. Jacobs, North Slope crude oil and dispersed ANSC crude to R.F. Sis, 1981 . Histopathological surveys of ovaries from petroleum production and control sites in the Gulf of Mexico. *J. Fish Biol.*, 18: 264-269.
- Youhnovski N, Dandarov K, Guggisberg A, and Hesse M (1999): Macrocyclic spermine alcholoids from *Verbascum*: Isolation, structure elucidation and synthesis of the (E/Z)- isomeric pairs(S)-verbasikrine/(S)-isoverbasikrine and (S)-verbamekrine/(S)-soverbamekrine. *Helv Chim Acta* 82: 1185–1194.
- Youhnovski N, Dandarov K, Guggisberg A, and Hesse M (1999): Macrocyclic spermine alcholoids from *Verbascum*: Isolation, structure elucidation and synthesis of the (E/Z)-isomeric pairs(S)-verbasikrine/(S)-isoverbasikrine and (S)-verbamekrine/(S)-isoverbamekrine. *Helv Chim Acta* 82: 1185–1194.
- Zeybek N (1985): Pharmaceutical Botany (in Turkish). *E.Ü. Eczacılık Fak. Yayınları*, No. 1. Bornova I zmir, E.Ü. Basımevi.