Bull. Inst. Oceanogr. & Fish., ARE, 14 (3) 1988. 101 - 110.

HAEMATOLOGICAL AND PHYSIOLOGICAL CHANGES INDUCED BY HEXAVALENT CHROMIUM IN *Clarias lazera*

A. M. HILMY, N. A. EL - DOMIATY, F. I. MOUSSA AND A. A. EL-MASRY.

Department of Zoology, Faculty of Science, Alexandria University, Egypt.

ABSTRACT

Haematological and physiological changes were studied in the blood of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L, as indictors of toxicity.

Pronounced decrease in R.B.Cs., haemoglobin and haematocrit were noted. This could be a result of erythropoiesis depression due to renal toxicity.

Higher total leucocytic count and fluctuations in their differential counts were observed. These changes indicte that a state of stress was present. The hyperproteinemia recorded was attributed to leakage of the albumin molecules through the damaged kidney. Results showed elevated levels in serum urea, uric acid and creatinin a reflection to renal failure. The hyperlipidaemia and hypertriglyceridaemia observed indicate a disturbance in the metabolism of these biomolecules. Elevated serum glucose levels recorded was interpreted on the basis of reduced glomerular filteration rate induced by chromium.

INTRODUCTION

Chromium is an essential element to animals and plants. Interest in chromium as an essential trace element is increasing rapidly. The landmark studies of Schroeder and his associates (1962,1970 and 1974) and of Mertz and his associates (1959, 1967 and 1978) established the presence of significant quantities of chromium in biologic fluids and tissues.

Chromium and its compounds are used in various branches of industry as fundamental or additive raw material and its salts are being discharged in over-increasing amounts into the aquatic environment. Because of its widespread use and toxicity, chromium can be a hazard to aquatic life.

Short or long-term exposure to heavy metals is known to cause a variety of physiological effects in fishes (Katz, 1975). Because of the dearth of background information for the effect of chromium on the haematology and physiology of the blood of the catfish **Clarias lazera**, it was hoped

to give an account of the changes occurring in a number of blood constituents of this species concerning four cellular (R.B.Cs., haemoglobin, haematocrit, total leucocytic and differential counts) and seven non-cellular parameters (serum total proteins, urea, uric acid, creatinin, total lipids, triglycerides and glucose) under acute chromium exposure.

MATERIALS AND METHODS

Tests were initiated with **Clarias lazera** as juveniles of 230 ± 10 mm. length and 130 ± 6.4 g weight. Fish were exposed to environmental chromium, at a concentration of 91.2 mg Cr^{+6}/l (96 hr TLm value) in tanks containing 40 l of aged tap water. Chromium was added in the form of potassium dichromate. Ten individuals were used in each test tank and ten in the control.

Samples of blood were withdrawn from the test fish after being removed from test aquaria without any type of anaesthesia, by transection of the cadual peduncle, about 1 to 1.5 ml of blood were collected from a single individual. Blood collected for cellular determinations was treated with anticoagulant and held in centrifuge tubes to be immediately analysed. Blood samples collected for serum determinations were drawn and centrifuged at 8000 rpm for 3-5 minutes. Packed cells were discarded and serum was stored under refrigeration until time of analysis.

Erythrocyte counts were made using techniques suggested by Hesser (1960) using Hendricks solution as diluent.

Total leucocytic counts were carried out using Shaw's solution (Shaw, 1930) and their differential counts were made on blood smears stained with May-Gimsas stain.

Standard precedures were adopted for estimation of haemoglobin (Mattenheimar, 1970), haematecrit (Wintrobe, 1974), and total proteins (Barnett and Youden, 1970), urea (Kaplan, 1965), uric acid (Ratliff et al., 1973), creatinine (Varley, 1963), total lipids, Farstad (1966), triglycerides (Rice, 1970) and glucose (Clerch and Miale, 1971) in serum.

All the results were statistically treated using the Arkin and Colten formulae (1963).

RESULTS

The present study reports on changes in selected blood components of a freshwater teleost, **Clarias lazera**, after acute exposure to chromium.

Erythrocyte count, haemoglobin content and haematocrit levels were marked by decreased values in all lots of fish exposed to chromium than the controls, (Fig. 1).

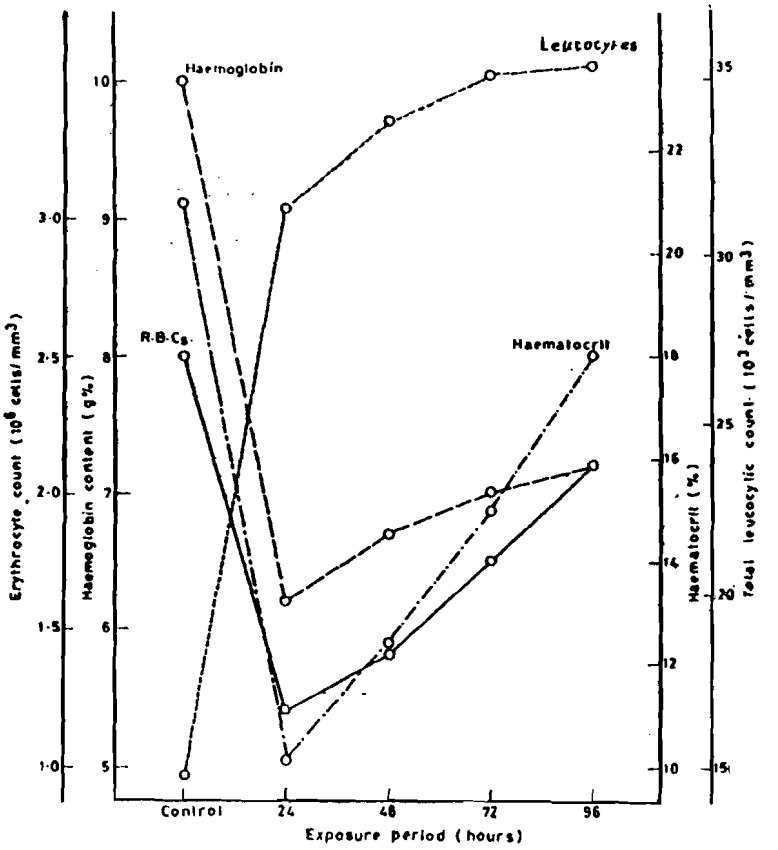

Fig. (1)
Changes in erythrocyte count, haemoglobin content, haematocrit value and total leucocytic count of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L for 96 hr.

Exposure of **Clarias lazera** to the toxicant caused significant increases in total leucocytic count (mean value was 14.8 x 10^3 white blood cell for control and 35.4 x 10^3 after 96 hr) and fluctuations in their differentials, (Fig. 2).

Mean total protein values are consistently lower than those reported for corresponding controls allover the experimental period (serum total proteins of experimental controls showed a mean of 2.75 g/dl while that of chromium treated fish was 1.1 g/dl after 96 hr, Fig. 3).

Experimental data indicated that the three non protein nitrogen fractions studied (urea, uric acid and creatinin) increased significantly as a result of treatment with chromium, (Fig. 4).

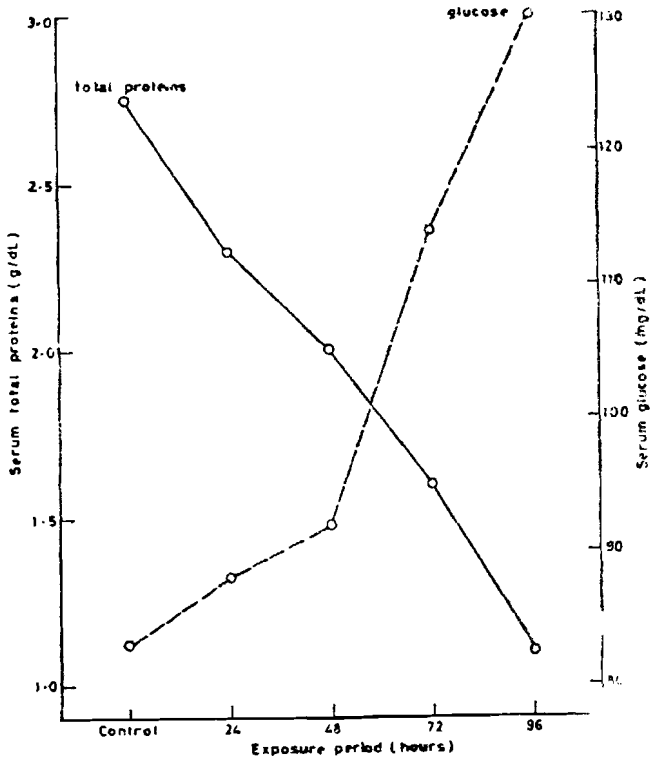

Fig. (3)
Serum total proteins and glucose concentrations of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L for 96 hr.

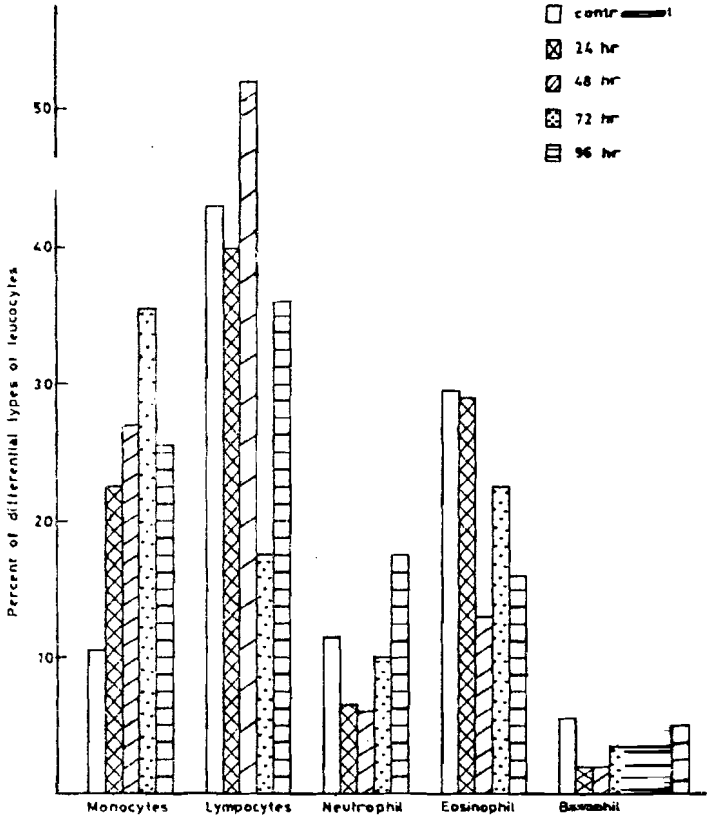

Fig. (2)
Ditterential count of leucocytes of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L for 96 hr.

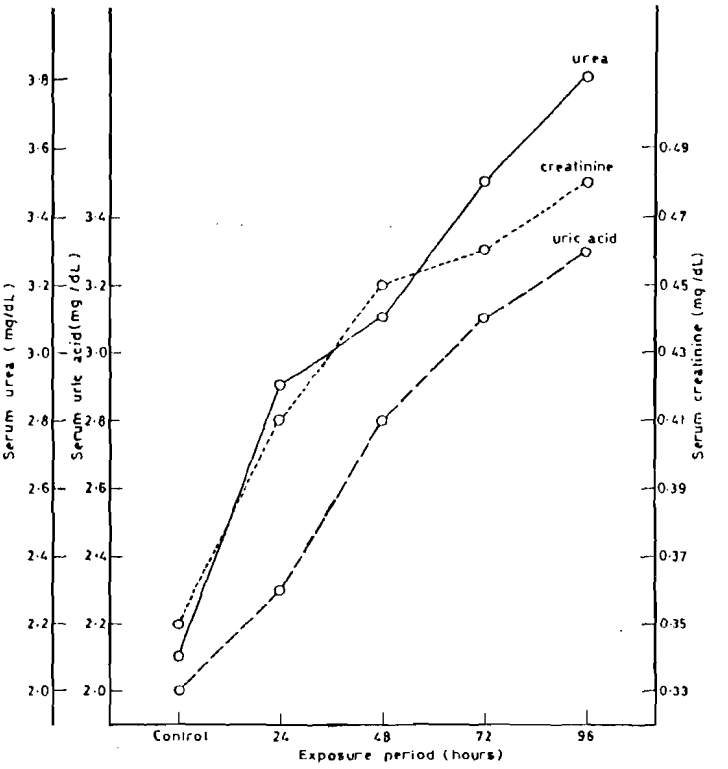

Fig. (4)
Serum urea, uric acid and creatinine concentration of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L for 96 hr.

The level of serum total lipid and triglycerides showed a general elevated trend throughout the exposure period. From a level of 208 mg/dl for total lipid and 46.5 mg/dl for triglycerides reported for controls, the concentrations increased to 427 and 72.4 mg/dl for the two parameters respectively at the end of experiments, (Fig. 5).

Serum hyperglycaemia developed within 24 hr after exposure to chromium (mean: 88 mg/dl). Means for all groups sampled between 24 and 96 hr tended to be higher than that of control value, (Fig. 3).

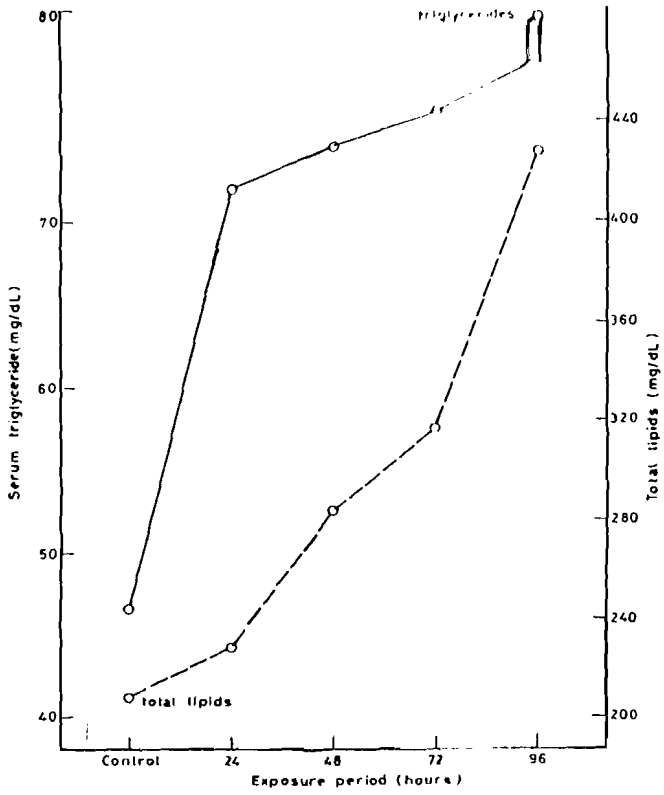

Fig. (5)
Serum total lipids and triglyceride concentrations of **Clarias lazera** exposed to 91.2 mg Cr^{+6}/L for 96 hr.

DISCUSSION

Haematological data indicated a significant decrease in erythrocyte count, haemoglobin content and haematocrit value after acute exposure of the fish under study to chromium. Hexavalent chromium compounds are well recognised as nephrotoxic and hepatotoxic agents. These effects on blood and circulation may contribute to the observed anaemia.

The results of chromium uptake (unpublished data) indicated that the kidney accumulated pronounced amounts of chromium when compared with controls. This may cause erythropoiesis depression, probably due to impaired erythropoietic stimulation.

In a contradictory condition to our results, Schiffman and Fromm (1959) noticed an increase in the number of erythrocytes, haemoglobin and percent of haematocrit in the circulating blood of rainbow trout **Salmo gairdneri.** after chromium exposure. Elevated values of total red blood cells and haematocrit were recognized by Srivastava et al. (1979) for a fresh water teleost **Colisa fasciatus** following acute exposure to chromium. Exposure of rainbow trout **(Salmo gairdneri)** to sublethal concentration of chromium, also induced pronounced increase in the population of circulating erythrocytes and haematocrit (Van Der Putte et al. 1982).

On the other hand, Tandon et al. (1978) working on rabbits, found that haemoglobin remained within the normal range in control animals and animals injected with chromium.

Chromium in toxicated **Clarias lazera** exhibited higher leucocytic counts than corresponding conts.

Conversly, a decrease in total leucocytic count has been recorded Srivastava et al. (1979) for the fresh water teleost, **Colisa fasciatus** exposed to chromium.

A fluctuation in the relative number of leucocytes was evident in **Clarias lazera,** throughout the experimental period. We have no plausible explanation of this. However, one may conclude that the changes observed in total leucocytic and differential counts during the present investigation indicate that a state of stress was present.

In the work desig.ed here, a significant decrease was observed in serum total proteins after exposure of fish to chromium. Hyperproteinemia is ecountered in many unrelated disease states. In nephrotic syndrome large masses of albumin may be lost in the urine as a result of leakage of the albumin molecules through the damaged kidney. Numerous clinical reports have described renal failure after oral ingestion of chromate (Fristedt et al., 1965 and Kaufman et al., 1970). In humans, the tubules are the primary site of injury, whereas in animals glomerular as well as tubular damage has been produced (Galloro et al., 1933). Pathological changes in the kidneys which have been observed previously in chromium exposed animals may explain the cause of the decline in serum total proteins recorded during this study.

The present results indicated that serum non protein nitrogen fractions studied (urea, uric acid and creatinine) were also disturbed. They showed elevated levels due to chromium exposure, an indication of renal failure. A similar increase in the blood urea level has been reported by Tandon et al. (1978) in rabbits treated with chromium.

Experimental acute renal failure induced by poisoning with chromate was previously reported by Berndt (1976), Biber et al. (1968), Franchini et al. (1978) and Kirschbaum et al. (1981).

Elevation in the serum total lipids and triglyceride levels of chromium treated fish has been found in the present work. Similarly Tandon et al. (1978) observed a marked elevation in the blood lipid levels of male albino rabbits injected with chromium.

The alterations in serum lipids in the experimental animals clearly indicate that chromium disturbs the metabolism of these biomolecules. This metal affect the fat metabolism by Other investigators (Mikailov et al., 1967 & Browning, 1969) have also shown that this metal affect the fat metabolism.

An experimental study of chromium poisoning in rabbits demonstrated liver cell degeneration, inflammatory cell infiltration, Kupffer cell proliferatio, and large amounts of chromium in the liver (Bianchini, 1941). Since lipid metabolism is primarily a function of liver, so the elevation of lipids than controls may be attributed to that chromium causes disturbances in the liver and enhances lipid production.

In these experiments it was shown that acute chromium poisoning caused significant elevation of serum glucose level. This result is consistent with the finding of Sastry and Tyagi (1982) for the fish **Channa punctatus** exposed to chromium. Increased blood glucose level has also been noticed in the freshwater prawn **Macrobrachium lamarrei** following chromium toxicity (Murti et al., 1983).

Chromium poisoning has been used as an experimental model of acute renal failure. Reduced glomerular filtration rate caused by chromium was previously recorded by Franchini et al., (1978). The observed hyperglycaemia in the investigation designed here may therefore be due to a decreased glomerular filtration rate as a result of renal failure induced in animals exposed to chromium.

REFERENCES

Arkin H. and R.R. Colton, 1963. **Statistical Methods, 4th ed.** Bermes and Noble, New York.

Barnett R. and W. Youden, 1970. A revised scheme for the comparison of quantitative methods. **Amer. H; Clin. Path.** 54, 454.

Berndt W. O., 1976. Renal chromium accumulation and its relationship to chromium-induced nephrotoxicity. **J. Toxicol. Environ. Health.** 1: 449-459.

Bianchini G., 1941. Sulla intossicazione da cromo, Turin. **Attualita di Scienze Mediche.** p. 48.

Biber ,T.U.L., M. Mylle; A.D. Baines; C.W. Gottschalk; J.R. Oliver and M.C. Mac Dowell, 1968. The study by micropuncture and microdissection of acute renal damage in rats. **Amer. J. Med.** 44: 664-705.

Browning E., 1969. **Toxicity of Industrial Metals.** 2nd ed.,Butterworth, London. pp. 119-131. **Butterworth,** London.

Clerch A. and J. Miale, 1971. A comparison of the unitest system with three other methods for determining blood glucose. **Am. J. Clin. Path.** 55: 159.

Farstad M., 1966. Determination of total lipids and the lipids of lipoprotein fractions of serum. **Clin. Chim. Acta** 14: 341.

Franchini I., A. Mutti; A. Cavatorta; A. Corradi; A. Cost; G. Olivetti and A. Berghetti, 1978. Nephrotoxicity of chromium. **Contr. Nephrol.**, 10: 98-110.

Fristedt B., B. Lindquist; A. Schutz and P. Ovrum, 1965. Survival in a case of acute oral chromic acid poisoning with acute renal failure treated by haemodialysis. **Acta Med. Scand.**, 177: 153.

Galloro B., W.C. Hunter and J.M. Roberts, 1933. Study of the effects of potassium bichromate on the monkeys kidney. **Am. J. Path.** 9: 133-148.

Hesser E.F., 1960. Methods for routine fish haematology. **Prog. Fish. Cult.**22, 164-171.

Kaplan A., 1965. Urea nitrogen and urinary ammonia, Standard Meth. **Clin. Chem.** 5, 245.

Katz M., 1975. The effects of heavy metals on fish and aquatic organisms. In P.A. Krenked (ed.): **Heavy metals in the aquatic environment.** New York. Pergamon Press.

Kaufman D.B., W. Di Nicola and R. McIntosh, 1970. Acute potassium dichromate poisoning. **Am. J. Dis. Child.**119, 374.

Kirschbaum B.B., F.M. Sprinkel and D.E. Oken, 1981. Proximal tubule brush border alterations during the course of chromate nephropathy. **Toxical. Appl. Pharmacel.** 58: 19-30.

Mattonheimer H., 1970. Micromethods for the clinical and biochemical laboratory. **Ann Arbor Science, Ann Arbor.**

Mertz W., 1967. Biologic role of Chromium. **Fed. Proc.** 26: 186 - 193.

Mertz W. and K. Schwarz, 1959. Relation of glucose tolerance factor to impaired glucose tolerance in rates on stock diets. **Am. J. Physiol.** 196: 615-618.

Mertz W., R.A. Anderson; W.R. Wolf and E.E. Roginshi, 1978. Progress of chromium nutrition research. In Kirchgessner M. (ed.). **Trace element metabolism in man and animals-** 3. Freising- Weihenstephan: Institute fur Ernahrungsphysiologie,

Mertz W., R.A. Anderson; W.R. Wolf and E.E. Roginshi, 1978. Progress of chromium nutrition research. In Kirchgessner M. (ed.). **Trace element metabolism in man and animals-** 3. Freising- Weihenstephan: Institute fur Ernahrungsphysiologie, Technische Universital Munchen, Germany. pp. 272-278.

Mikhailov V.A., K.N. Klychima; L.N. Belyaeva; M.S. Sadilova; T.S. Kazantseva; E.S. Trop; A.M. Kleiner and G.W. Belobragiva, 1967. The results of and prospects for studies of the pathogenesis, therapy and individual prophylaxis of industrial poisoning with manganese, chromium and fluorine compounds. **Gen. Probl. Ind. Toxicol.**Moscow 124-127; (1968) Biol. Abstr. 49, 40285.

Murti R., Omkar and G.S. Shukla, 1983. Chromium toxicity to a freshwater prawn, **Macrobrachium lamarrei** (H. M. Edwards) **Toxicology letters** 18: 257-261.

Ratliff C.R. and F.F. Hall, 1973. Laboratory Manual of Clinical Biochemistry. Vol.I. **Scott and White Memorial Hospital Publications Office,** Temple,Texas.

Rice E., 1970. Triglycerides (neutral fat) in serum. Stand. Meth. **Clin. Chem.** Vol. 6 Academic Press, New York.

Sastry K.V. and S. Tyagi, 1982. Toxic effects of chromium in a freshwater teleost fish, **Channa punctatus. Toxicology letters.**11: 17-21.

Schiffman R.H. and P.O. From, 1959. Chromium-induced changes in the blood of rainbow trout, **Salmo gairdneri. Sewage and Industrial Wastes.** 31:205-11.

Schroeder H.A., 1974. The role of trace elements in cardiovascular **diseases. Med. Clin. North Am.** 58:381-396.

Schroeder H.A., J.J. Balassa and I.H. Tipton, 1962. Abnormal trace metals in man-chromium. **J. Chron. Dis.** 15: 941-964.

Schroeder H.A., A.P. Hason and I.H. Tipton, 1970. Chromium deficiency as a factor in atherosclerosis. **J. Chron. Dis.** 23,-123-142.

Shaw A.E., 1930. A direct method for counting the leucocytes and erythrocytes of bird blood. **J. Path. Bact.** 32: 833-835.

Srivastava A.K., S.J. Agrawal and H.S. Chaudhry, 1979. Effects of chromium on the blood of a freshwater teleost. **Ectetoxicol.Environm. Safety.** 3: 321-324.

Tandon S.K., D.K. Saxena; J.S. Gauer and S.V. Chandra, 1978. Comparative toxicity of trivalent and hexavalent chromium; alterations in blood and liver. **Environ. Res.** 15: 90-99.

Van Der Putte I., W. Van Der Galien and J.J.T.W.A. Strick, 1982. Effects of hexavalent chromium in rainbow trout **(Salmo gairdneri)**after prolonged exposure at tow different pH levels. 6: 246-257.

Varley H., 1963. **Practical Clinical Biochemistry**. Heinmann Medical Books,London.

Wintrobe M.M., 1974. **Clinical haematology.** Lea and Febiger. Philadelphia, PA. 1896 p.