THE EFFECT OF TEMPERATURE ON THE TOXICITY OF PETROLEUM AND POTASSIUM DICHROMATE ON THE TILAPIA *Sarotherodon vulcani*

by

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This is to certify that this undergrduate thesis entitled "The Effect of Temperature on the Toxicity of Petroleum and Potassium Dichromate on the Tilapia Sarothenodon vulcani and submitted by Noel Fermindoza to fulfill part of the requirement for the degree of Bachelor of Science in Biology was submitted in March ,1991.

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Sublethal doses of petroleum (1 ppm) and potassium dichromate (130 ppm) are mixed in two separate tanks. Sarothenodon vulcani are placed in the 2 tanks for 24, 48, 72, hrs. Temperatures are then increased in the 2 tanks. By counting mortalities and using statistical test (L=.10) prove that an increase in temperature causes an increase in the toxicity of the 2 pollutants in S. vulcani. The induced effect of the temparatures potentiates the lethal effect of each pollutants. Histological findings reveal that a temperature aggrevates the lethal effects of the 2 pollutants in terms of the cellular (especially in muscles and gills) and physiological processes in S. vulcani. As an offshoot this affects the behavioural patterns of the fish under stress. In temperatures 30o - 38o C , lethality in petroleum increasess 13 times a 100 % kill, while on potassium dichromate mortality increases 6 times a 100 % kill at temperatures 32o - 38o C. Since optimal temperature of S. vulcani is from 14oC - 42oC, sublethal concentration of pollutants lowers the optimal temperature and degree of survival. PH and dissolve oxygen (DO) play a part in these effects since with the presence of pollutants the PH is affected while the presence of increasing temperature the solubility of oxygen is changed. This paper describes the ill- effects of pollution and recommends that " palay-isdaan" farmers prevent any of these pollutants from entering the physiological systems of their cultures.

INTRODUCTION

Man's modern society gave rise to one basic menace - pollution. These pollutions whether in land, air and sea changed man's environment and eventually altered his ecosystem. One such pollutant are trace metals which can be zinc, cadium,copper and

chromium. These trace metals are frequently discharged in the environment by means of the exhaust in industrial factories.

In the past decades, organic chromium compounds gained attention as health hazards. Chromium is a white lustrous metal which can be highly polished. It tends to be brittle at low temperatures but it is highly resistant to oxidation and corrosion and so is widely used to provide a protective coating. Chromium metal and chromium compounds are produced from chromite and have general uses namely, metallurgical, refractory and chemical. In metallurgy, chromium is used in manufacture of chrome-steel or chrome-nickel-steel alloys for greatly increasing resistance and durability of metals and for chromium plating of other metals. Because of its high melting (1890 oC) it is used for manufacturing heat resistant and corrosion-resistant industrial materials.

Chemicals made from chromium are used as a tanning agent, dye-metallizing agent, pigments, mordants, catalyst, corrosion inhibitors, fungicide, wood preservatives, metal anoclizing agent and flare. Sodium radiochromate is medically used to evaluate the life span of red blood cells and also as a fecal marker. Potassium dichromate ($K_2Cr_2O_7$) is widely used in industry as oxidant and an analytical reagent. Aside from this,

it is widely used in galvanizing iron, printing in, synthesis in various pharmaceuticals, protochemicals, chrome-tanned leather, chrome alum, chrome pigments & ceramic products.

Potassium dichromate is readily soluble in water like sodium and ammonium dichromate. It is important to note that exposure to these inorganic chromium compounds maybe due to its considerable amounts present in the air. From the metallurgical industry chromium sesquioxide is probably the most abundant form of airborne travalent chromium. Hexavalent chromium in the air originates mainly from the production of chromate & dichromates by the chemical industry. Thus contamination of such compounds are due daily exposure as a result of occupation - such as spray painters applying paints containing chrome pigments, welders, metal cutters & scrap metal workers handling steel parts coated with such paints, aniline dye producers using chromates as oxidants, chrome platers & painters inhaling chrome pigment-containing inks discharged into the air of workrooms from rotary presses.

For non-occupationally exposed people, chromium enters their system due to the food, water & animal fats esp. butter. In the adult diet 30-200 ug, of w/c 6-10 ug could be contributed by drinking water. It must be noted that variations exist in the amount of protein sources for instance liver & eggs have high chromium contents while fish has a low chromium content. The

drinking water as 50 ug per kg. body weight.

Another type of pollutant is oil. Among the derivatives of oil, petroleum is the most accessible to human and marine life. The exhaust coming from factories, turbines and automobiles etc. either on air or on land affect man's physiological function & promotes respiratory sickness.

In March 16, 1978, the supertanker Amoco Cadiz off Brittany produced the biggest discharge of petroleum in maritime history. These cases give adequate proof that an era of oil pollution in the ocean seas to have begun. In the attempt to frame the effects of petroleum on marine organism as well as freshwater forms an important concept is introduced ------- STRESS. This is a major factor first enunciated by Hans Selye, a Canadian in 1936, this concept constitutes 3 phases: alarm, resistance & exhaustion.

1. alarm ---------- (adaptation)	2. resistance ---------- failure of	3. exhaustion
short term responses: behavioural and other responses to non-optimum environment	long term responses: adaptive enzymes protein bending of metals, induction of mixed-function oxidases, population selective action favoring survival of resistant individuals changes in reproductive strategy	critical biochemical functions leading to functional disorders and death, gradual diminution and disappearances of some populations due to reproductive failure.

Selye summed up stress as "the sum of all physiological responses

by which an animal tries to maintain or reestablish a normal metabolism in the face of a physical or chemical force.

Brett in 1958, redefined this as a "state produced by any environmental or other factor which extends the adaptive response of an animal beyond the normal range or which disturbs the normal functioning to such an extent that, in either case, the chances of survival are significantly reduced. Another definition is that it is the effect of any force which tends to extend any homeostatic or stabilizing process beyond its normal limit at any level of biological organization. In essence the stress depicted represents the sum of myophological, physiological, biochemical & behavioural changes in individuals that result from the actions of stressors.

Petroleum contamination of estuarine or coastal waters, either as an acute episode (an oil spill) or as a chronic stressor, clearly affects marine organisms. Toxicity is most destructive during the early life stages of fishes upon contact with oil. Sensitivity can vary in differenct species & would depend on the extent of previous contact of the parent population with oil contamination (in areas of chronic spills or seeps). Adult animals may exhibit many physiological, biochemical and behavioural responses to oil contamination. It must be noted also that there also exist some histopathological signs, modified enzyme activity altered reproductive behaviour and in extreme cases, mortalities occur.

This stress concept can be applied also to the mode of action of potassium dichromate. Since chromium has 2 forms , the hexavalent type is more toxic than the trivalent one. In mammalian metabolism, chromium compounds enter the body through the pulmonary or gastrointestinal routes and in hexavalent forms the skin serves as an entry point. Once found out to occur in large amounts would result to chromium poisoning. This is seen in hexavalent chromium w/c was found to cause severe inflamamation of the digestive tract w/ consequent necrosis & even perforation as well as immediate necrosis of hepatic & renal tissues. These reactions could lead to rapid death as a result of cardiovascular collapse.

Chromium overdose also associated w/ respiratory cancer which is easily accessible to workers from chrome-pigment industry. Other "exhaustion" results of stress in mammalian organism would include bronchial carcenoma, laryngeal cancer and other complication that would result from the destruction of the cells (squamous & round) of the respiratory tract.

In fishes, the hexavalent form of potassium dichromate would induce the destruction of the gills, kidney and gut. For mollusca, many reports indicate localization of this heavy metal on the kidney. As for crustaceans, these would be accumulated in the hepatopancreas, gills & green glands. Some like the Ostrea accumulate this in their amebocytes & in blood cells.

This paper aims to determine the extent of toxicity of Potassium chromate and Petroleum, which is a derivative of crude oil, to the tilapia (Sarothenodon vulcani) in respect to temperature. It aims to trace the temperature tolerance of the tilapia given a constant hardess, feeding habits and growth capacity in response to the various "stressors" being added. It also would like to study the effect of varying the temperatures of these "stressors" to the morphological, behavioural and as well as histopathological aspect of the tilapia. Thus in essence it would try to determine the correct concentration of a metal (potassium dichromate) and a hydrocarbon (petroleum) to determine the survival rate of the tilapia given a certain temperature.

Practicality is the main concern of this paper since it would offer an overall effect of the damage done by 2 basic pollutants in the world. It is a known fact that potassium dichromate is extruded by large factories either into the air or nearby bodies of water. On the other hand, petroleum is frequent in oceans where oil spills occur. It is a fact that factories extrude also the by-product and derivatives of petroleum in the air or in certain bodies of water. Thus, the need for studying both its effect on Tilapia (Sarothenodon vulcani)one of the most staple foods of Filipinos and a reliable export product of the country, is imperative.

The results of this study would be beneficial to the

country's agri-aquaculture or "palay-isdaan" program since it would clearly demonstrate the effects of certain pollutants on their yield at a given concentration. In Manila as well as in provinces, one could see some partial land used for "palay-isdaan" culture situated in between large metal factories, printing presses and, oil refineries, packaging plants etc. This study would increase the awareness of the farmers of the danger posed by these factories to their yield. On the other hand, the buying public and the tilapia-lovers of this country would be aware of the diseases which would result as a complication of eating contaminated tilapia or any other organism.

Aside from awareness of the effects of these pollutants, the result of this study would enable the farmers to increase their tilapia culture in the "palay-isdaan". The farmers would know the right concentration of potassium dichromate in their fertilizers w/c could not harm the tilapia living in their plot. Secondly, the farmers would think twice of spraying pesticide and other chemicals for they too have a concentration of trace metals like potassium dichromate. Third, it would give them the necessary information of how their tractors and other agricultural machinery could ruin their yield due to leakages in oil or petroleum in their plot. Thus, the farmers would be reminded of the value of land conservation.

Lastly this paper would justify the ill-effects of

pollution either in the air, land and especially the seas. Since the Tilapia is a fish which has a high tolerance level, it is safe to correlate the effects of these pollutants to other marine organisms. This would further discourage the long stigma which have plagued human modern society ---------- Pollution.

REVIEW OF RELATED LITRATURE

Tilapias are important products in aquaculture of tropical & subtropical countries because it serves as a source of protein for the population. It is used in aquaculture since it is resistant to diseases, can easily adapt to any varying degree of hardness of water, can produce high yield and be able to survive at very low oxygen tensions. They could also survive on a wide range of foods both artificial or natural (even manure) & can withstand a wide range of salinities. Aside from this it has a firm white flesh and no intermuscular bones thus making it an excellent table fish.

The tilapias belong to the family of Cichlidae, with about 700 species and is actually distributed to countries like Africa, Central America up to Mexico, the northern half of South America and a part of India. This fish is divided into two genera according to their behaviour. The substrate breeders retain the generic name Tilapia; while the mouthbrooders have been termed as

the genus Sarothenodon. A classification of Tilapias according to breeding behaviour results in 4 groups.

1.) Substrate breeders

2.) Maternal Mouthbrooders, including all species of Sarothenodon

3.) The only known paternal mouthbrooders, S. melanotheron, previously referred to as T. macrocephala and S. heudeloti.

4.) The known bi parental mouthbrooder S. galilaeus.

Chromium Toxicity

Chromium can be of the hexavalent or the trivalent type. Chronic toxicity was observed in several mammalian species with Cr+6 in concentrations more than 5 ppm in drinking water. In rabbits, it caused subacute toxicity & no growth rates. Dogs tolerate up to 11.2 ppm of Cr+6 in water for 4 years without any ill effects. It is therefore noted that chromate and dichromates are relatively more toxic than the trivalent form because the hexavalent forms would easily penetrate the cell membranes easily and are strong oxidizing agents.

The three hexavelent chromium compounds - potassium dichromate, sodium dichromate and chromium trioxide - are included among the toxic and hazardous industrial chemicals listed by the International Technical Information Institute. In man, the minimum or threshold concentration can be exposed to

these compounds in the air is 0.1 mg per m3 (as Cr+3). Leonard and Lawvery's finding reveal also that in human cases of chromium poisoning could cause severe inflamation of the digestive tract w/ consequent necrosis and even perforations of the hepatic & renal tissues. It could also lead into the perforation of the nasal septum, orthoergic & allergic contact dematitis and irritation of the conjunctiva upon inhalation or contact of these compounds. Further exposure would lead into the development of chronic rhinitis, sinusitis, hydrops of the nasal sinuses, nasal mucosal polyps, inflammatory & ulcerative conditions of the gastrointestinal tract and often an unbalanced ratio of the formed elements of the blood leading to an increased time for wound healing and longer bleeding time of wounds. This attack on the nasal mucosa and respiratory tract could be aggrevated and eventually would develop into respiratory cancer (esp. lung & laryngeal cancer). Since it also attacks the gastrointestinal mucosa it could also be a cause of gastrointestinal cancer if exposed to a greater degree.

Potassium dichromate is readily soluble in water as are sodium and ammonium dichromate. Like in mammals according the California State Water Pollution Control Board in 1954, the hexavalent chromium is more toxic to fishes compared to the trivalent form. It is also emphasized that the reduction or transformation of the hexavalent form to a trivalent either by heat, use of organic matter & reducing agents should not in-

crease the toxicity to fish.

It is found out that the toxic level of potassium dichromate ($K_2Cr_2O_7$) varies in species. A.B. Southgate in 1948 proposed a range of 5.2 ppm (concentration of chromium) onwards toxic to brown trout. M. M. Ellis in 1937 reported that the toxicity of chromium for goldfish is 117 ppm. J. Grinley in 1945 also reported a concentration of 57 ppm $K_2Cr_2O_7$ caused rainbow trout to lose their equilibrium in 60 hrs. pH 5.5. Unfortunately, there was no data included in these controls and all were conducted using distilled water thus proving harmful to these test fishes.

In 1948, C.W. Klassen et al reported a concentration of 50 ppm was toxic for a week's exposure. The experiment was conducted with a total hardness of water of 294 ppm. Total hardness is a characteristic of water impurity. This is caused by the presence of dissolved mineral salts, which would reduce the cleansing power of soap and would form suds. This characteristic would vary from the different rivers and seas around the world. In Klassen et al experiment, there is a discrepancy (although small) would be attributed to the variation in ammonia and pH present in the water used.

For eels, S. Oshima in 1931 reported that it would need 0.005 M $K_2Cr_2O_7$ solution for it to survive 5.35 hrs. But it was refuted by an experiment in which there were no eels killed in 50

hrs. using 0.0005 M $K_2Cr_2O_7$. This finding would be supported by the findings of L. Leger who in 1912 reported that there is a thing of decreased toxicity of potassuim dichromate. He pointed out that cation antagonism is one of the major causes of the difference. Thus with this concept, it can be supported that this cation antagonism is dependent on the hardness of the water and the presence of calcium as founded by L.V. Heiburn in 1952.

Petroleum Toxicity

There are many cases of hydrocarbon pollution. But the most common of all is in the form man has been "accustomed" to - an oil spill. It is believed millions of tons of hydrocarbons are releaased into the oceans annually. This release is slow, deliberate & chronic arising from man's activity which accounts for 90% of the oil going into the sea each year. This release comes from rivers (domestic or industrial origin), coastal refineries, dumping or deballasting of tankers at sea and search for offshore oil. Another source is atmospheric fallout, estimated at 600,000 tons per yer originating in land based discharge of oil. Since petroleum is a derivative of oil, it must be important to know the different processes which take place when hydrocarbons are ejected in the marine environment.

Fate of Hydrocarbons in Marine Environment

1. Spreading, evaporation, dispersion

Upon ejection, hydrocarbon spreads rapidly over the surface and lose its volatile components by evaporation & dispersion in the marine environment. These 3 processes depend not only from the physical properties (density, viscosity, surface tension etc.) and chemical properties (composition, reactivity) of the oil, but also on the amount of energy available in the environment. This energy can be mechanical (wind, waves, current) or thermal (water, hydrocarbon and air temperatures).

The spreading of the hydrocarbons entails the formation of surface film which contains the lightest components of the hydrocarbon. This surface film would allow the longer time for the evaporation, modify sea swell and obstruct the mechanical action of waves. A number of chemical pollutants (organic chlorides, detergents, pesticides, etc.) dissolve easily in this film which are retained and concentrated. Chemical pollutants (heavy metals) or biological pollutants (bacteria, viruses) are pitched up by polar absorption. These pollutants and other substances evolved due to this process are transported to different parts of the marine environment.

2. Emulsification.

This occurs during oil spillage wherein 2 emulsions are formed in the water. The oil-in-water emulsion is formed on the surface and then dispersed by currents & waves. The other is a water-in-oil emulsion called "mousse". This emulsion seep below the water and could destroy marine life. Ordinarily, man deviced

chemical surface-active-agents to break up this emulsion but unfortunately such chemicals allow the further infiltration of oil deeply and contaminate burrowing species.

3. Formation of tar lumps

Hydrocarbons which had stayed in the water for a longer period of time and which were not degraded by the first 2 processes tend to form stabilized agglomerates (tar lumps). These tar lumps consist of heavy hydrocarbons, oxygen, nitrogen or sulfur compounds and mineral compounds (35%), especially iron oxide. This would accumulate in living organisms or in the mobile substrata.

4. Chemical degradation

Hydrocarbons which would remain from for few weeks to several years undergo chemical degradation mainly by photo-oxidation. This is initiated by primary photolysis which gives rise to free, highly reactive radicals which recombine into a large number of chemical compounds like polymers, oxidation products (alcohols, aldehydes, ketones) etc. Sometimes these chemicals have been proven harmful to organisms.

5. Biodegration

Microorganisms play an active role in this process. This process is dependent upon the type of microorganisms, mineral salt composition, chemical concentration, presence of supplementary nutrients, oxygen concentration, temperature and

pH. It must be also noted that the rate of degradation is dependent upon the molecular configuration of the hydrocarbon. Thus, straight-chain alkanes (paraffin hydrocabons) are more easily degraded.

It is possible since some bacterial strains (hydrocarbon utilizing) degrade hydrocarbons, it is also possible that some only store it but not transform it. This proves to be a threat to the ecosystem since some poisonous properties are retained and in most cases would affect all organisms due to the process of biological amplification. This is proven to the fact that zooplankton and most mollusca accumulate hydrocarbons near an oil spill of Annoco Cadiz near North Brittany shore lines and was found out that 3 to 10% of these hydrocarbons are present in their tissues.

6. Sedimentation

Hydrocarbons would seep into the ocean floor and would form potato-size lumps with sand. In some shorelines layers of "mousse" were buried up to 60 cm. These layers would mean a potential recontamination of intertidal areas. Some would be deposited to other tributaries of the sea like creeks, estuaries, lakes, etc. & could affect the dead or dying macro-vegetation which would eventually lead into the existing food chain.

The greatest damage from a petroleum spill is the near shore environment which would result in the deposition on and subsequent penetration into the sediments. The consequence of

this is the ingestion of toxic hydrocarbons by living organisms are almost instantaneous and near-total destruction of either poisoning, suffocation or asphyxia. These deaths would upset the ecological balance affecting other organisms belonging to the same major trophic level.

According to the Council on Environmental Quality (CEQ) of USA, plaice, sand-eels, gobies, shad, salmon and smelt are the fishes most vulnerable to hydrocarbons. Desaunay (1979) made works on fishes during the petroleum wreck of Amoco Cadiz and found out that integumental lessions such as fin erosion was found on plaice (Pleuronectes platessoa). In this fish, the posterior fins especially the areas in intimate contact with the bottom are eroded. He also found out in gadoid (Gadus mochua, Pollachius pollachius) the caudal fin was affected. An associated phemonenon was called "bent fin rays", which either accompanied or followed fin erosion. Ziskowski et al in 1980 found the disease signs of fin erosion and the bent fin ray condition from fishes collected from the highly contaminated New York Bight, the California coast, Puget Sound, Biscayne Bay, Escambia Bay, Irish Sea, Tokyo Bay & French Coast. He proposed that these integumental lessions are of 2 types.

a.) site-specific found especially in demersal fish

b.) generalized, found especially in pelagic fish; with involvement of all fins but especially the caudal, & with occasional infections. Fin erosion disease would then include

epidermal hyperplasia, dermal fibrous, hyperaemia, hemorrhage (occasional), no consistent bacterial infection and no pronounced inflammatory response. McDermott & Sherwood (1975) in California found DDT levels to be significantly higher in fish with fin erosion, and polychlorinated biphenyl (PCB) levels slightly higher in such fish than in normal individuals. Means & Sherwood (1974) found that when epithelial tissues are in direct contact with sulphides, heavy metals & chlorinated hydrocarbons could remove or modify the protective mucuos coat thus giving much proof in the case of fin erosion disease.

Soluble aromatic hydrocarbons can be lethal to adult animals in low concentrations (1-100 ppm), and more destructive to larval stage at even lower concentrations (0.1-1 ppm). Death has been used as a measure of biological response. It is a believed that these values are dependent upon the species of aquatic organisms. Apparently, crustaceans and certain benthic organisms are most sensitive to 1 to 10 ppm concentration, while fishes and bivalves are moderately sensitive to 10 to 100 ppm concentration.

Physiological or behavioural description could occur in adults could be seen when exposed in sublethal concentration of petroleum. This sublethal level would range from 10-100 ppb (parts per billion) & would depend on the species of an animal. Inhibition of mating response, reduced fecundity, chromosomal abnormalities and larval development can be observed. Its effect in animals and plants include: delayed cell division in

phytoplankton, abnormal fish spawn; reduction of chemotacting feeding responses in snails; inhibited mating responses of male crabs to sex phenomes' decreased filter feeding activity in mussels; and decreasing survival and fecundity in worms.

Aside from fin erosion disease, direct contact (coating) & ingestion of petroleum fishes suffer from occlusion of the gills or the digestive tract, disablement of appendages, irritation of mucuous membranes important to respiration. Exposure of fish & shellfish to as little as 1 ppb soluble petroleum components can lead to accumulation of potentially carcinogenic polycyclic aromatic fractions in marine food chains. According to Halstead, 12% of a sample of 16,000 sole from San Francisco Bay, presented an average of 33 tumors per fish in the vicinity of petrochemical waste disposal sites. Thus, transcient changes in the upper layers of the ocean and longer-lasting changes in the bottom sediments and associated benthic animals have been observed in the presence of petroleum spill or pollution.

The Role of Temperature

Temperature may be both and a controlling factor without a threshold. Every aquatic organism has a certain range of environmental temperatures that can be tolerated more or less indefinitely. This tolerance zone is determined by an interplay of developmental, genetic and environmental influences. It is determined by the stage of the organism's development, it's age,

physiological condition; stage in the life history. For example, juvenile stages may have a narrower zone than adult stages. The position of the tolerance zone in the thermal spectrum is often influenced by temperature accilation but ultimate limitations are fixed genetically. Thus by acclimation an organism to higher or lower temperature, the tolerance zone may be moved upward or downward respectively within the genetic limits of the species.

Temperatures above or below this zone tolerance are considered to be the zones of resistance. Hughes Roberts et al (1973) confirmed that aquatic animals such as the trout suffer from tissue anoxia at upper temperature levels. Since this is the case, the effects of any toxicant that either increases metabolic demand (e.g. copper) or blocks oxygen uptake at the gill level for fish (e.g. zinc) may be potentiated (rendered more active physiology) by increasing temperatures. Hochachka & Somero (1970) proposed that at high temperature cellular enzymes become inactivated. They supported this observation by stating that most cellular enzymes show sharp increases in the Michaelis-Menten constant at high & low temperatures. The Km is inversely proportional to the affinity of the enzyme, thus increasing the Km, the reaction catalyzed by the enzyme might decrease to a lethal level. On the other hand, Cairns et al (1964) ruled that thermal stress may induce release of coagulating enzyme.

The tilapia has a distinct preference for higher water temperatures. Reite et al experimentally obtained an upper lethal temperature of 40 oC & reported brood females as mobile bet. 40-44 oC. The normal water temperature range for tilapias is 20 to 30 oC but can withstand lower temperatures. The only species capable of surviving a 10 oC temperature are T. zillii, S. aureus & S. galileus at the northern limit of their distribution (Syria & Israel) & S. mossambius & T. spermanii at the southern limit of their distribution in Africa (Jubb - 1967). Nevertheless S. aureus (referred also as S. niloticus) is cold affected at 13 oC, while orientation of S. mossanbicus is disturbed at 11 oC.

Most tilapias do not eat or grow at temperatures below 15 oC (according to Bardach et al in 1972; Dendy et al in 1967) and do not spawn at temperature below 20 oC. The optimal temperature range is 26 to 29 oC for most species (acc. to Rothbard 1979). The only exception is T. spermanii which spawns at 16 oC. according to Spass (1960; cited by Balarin & Hatton 1979), the tilapia's optimum temperature for maximum growth is between 19 & 28 oC. On the other hand, Caulton (1975) believed that its preference is bet. the temperature range of 37 oC & 41 oC.

This study is a pioneer one since it tackles mainly the effect of 2 pollutants: potassium dichromate & petroleum in a certain temp. to the tilapia (Sarothenodon vulcani - formerly reffered as Tilapia niloticus) , a fish used in Philippine

aquaculture. No such study has been able to correlate the 2 pollutants with temperature and using tilapia at the same time as test organisms. John Cainrs Jr. & Arthur Scheir of the Academy of Natural Science of Philadelphia is in 1959 presented a study entitled "The Effects of Temperature & Hardness of Water Upon The Toxicity of Potassium Dichromate to the Common Bluegill Sunfish". It found out that the concentration which resulted in Lc50 or 50% death in 96 hrs. was 320 ppm. ion soft water at both 18 o and 30 oC, 382 ppm. in hard water at 18 oC and 369 ppm in hard water at 30 oC. Lc50 is the concentration of the toxicant which would cause a 50% mortality in a specific time and the soft & hard waters would refer to the amounts or concentration of dissolved ions in a certain amount of water. Thus, a hard water would be more polar than the soft one and would produce "suds" upon the addition of soap in the water. Some other pertinent works are mentioned earlier.

Regarding the effect of hydrocarbons such as petroleum, the previously stated are pertinent examples. But in a more recent study, Prasad, M.S. in 1989 made a study on the effects of crude oil on the air breating organs of the striped gourami (Colisa fasciatus). He found that when studied using SEM, there are pathological effects of crude oil on the air-breathing organs of the striped gourami. He found out that the buccal cavity is sensitive to crude oil & its derivatives than the epithelia of

the superbronchial chamber and the labyrinthine organ. Mucous cell heperplasia, shrinkage of the respiratory epithelius & telengiectasis of blood capillaries in the air-breathing organs can be seen. Cell hypertiophy, lessions and sloughing of air breathing epithelius increases as one would increase hydrocarbon concentration.

This study is congruent to the study made by Anderson et al in 1974 and Neff et al in 1976. They both described the histopathological consequences associated with the exposure of an euryhaline fish, Fundulus heteroclitus, to naphthalene, the most toxic PAH (polycyclic aromatic hydrocarbons) which are known components of crude & refined oil-water mixtures. Naphthalene concentration is at 0.002-30 mg l-1 for 15 days. The results were:

1.) Necrosis of neurosensory cells, particularly those of the olfactory and lateral line systems, occured at concentrations as low as 0.02 mg l-1.

2.) Small blood vessels were enlarged & blocked.

3.) Pathological lesions & necrosis were noted in the brain, liver and pancreas at .2 mg l-1.

4.) Gut mucusal necrosis and skeletal muscle degeneration occured.

5.) Napthalene was selectively accumulated in organs most susceptible to pathology.

6.) Gill hyperplasia & haemorrhage at higher concentrations.

Longwell in 1977, 1978 in his studies regarding the effect of petroleum spill on the mutagenity & chromosomal abnormalities of eggs pollock & cods resulted in the ff. findings:

1.) About 20% of cod eggs & 46% of pollock eggs are dead from the spill zone

2.) Pallock embryos were grossly malformed (18% of the eggs) at the edge of the slick & 9% were abnormal

3.) Eggs in the dead & morbid categories displayed a combination of cytological abnormality of the embryo's cells or of the nuclear configurations, coupled with division arrest.

It should be noted that these abnormalities of the embryos & larvae can be studies at a macroscopic level. Hawkes et al in 1980 found that these abnormalities include malformed jaws, flexuses of the vertebral column, lower embryonic heart rate, loss of coordination & equilibrium, and degeneration of neurosensory cells. So far, associations of chromosomal & gross abnormalities have not been proven.

MATERIALS AND METHODS

This research plan is divided into three parts. The first part is the research methodology which contains a description of the test organisms, instrumentations and the process of data collection and interpretation that was used. This would include a protocol regarding the histological examination of the gills. The second part deals with financial considerations

regarding the chemicals, instruments and test organisms used in data collection. Lastly, there is a timetable which the experimenter must undertake in order to complete the experiment.

The experimental set-up was conducted in the experimeter's residence from Dececber 19, 1990 to Jan. 18,1991. Histological preparations were conducted in the Philippine General Hospital from Jan. 28, 1991 to Feb. 6, 1991. Histological analysis of the gills and muscles were accomplished at the National Science and Research Institute at Diliman for a period of 5 days. Photomicrographs were also furnished during this period at the same institution. Thus, the experiment required a duration of 3 months from December of 1990 to Febuary of 1991.

Research Methodology

1. The test containers that were used are 5-gallon Pyrex jars. Each jar was fitted with an aeration tube (7 mm outside diameter) which extend the lenght of the jar, and a short air vent tube that also served as an aperture through which a short length of Tygon plastic tubing could be inserted for periodic sampling.

2. Constant temperature was maintained by immersing the test containers in a water-filled constant temperature vat in which a balanced heating - cooling system is in operation. It could also be maintained by using incandescent light bulbs on top

of the jars.

3. The waters used are prepared from distilled water and analar grade chemicals with total hardness of 2 . pH was maintained at 5.0 by appropraite dosing of H_2SO_4 and NaOH. Hardness is characteristic of the water due to the presence of dissolved mineral salts. The presence of this mineral salts would make the water form suds or precipitate upon application of cleansing soap. This degree of hardness is considered tolerable to the tilapias. The chemical composition of the dilution water is given at Appendix A.

4. The juvenile fishes (mean weight and range of length determined) of S. vulcani selected at random was initiated from the stock tank, and allowed to acclimatize to the test apparatus for 24 hours. The fish were fed daily with chopped, freshly cooked shrimp. In one container no single fish should vary more than 50 % in total length from any other fish.

5. The acclimatization process for fishes that will be tested for temperatures 40 degrees centrigrade above and 10 degrees below is slightly over two weeks . This is due to the fact that a temperature of 1 degree centigrade is increased and decreased per day starting from a 18 degree centigrade mark point. During the period of acclimatization if more than 5 % of the fish dies, the entire group is to be discarded.

6. Before raising the temperature, $K_2Cr_2O_7$ and petroleum

were introduced and mixed thoughly into the water in 2 separate containers . The concentrations introduced were 1 ppm (parts per million) for petroleum and 130 ppm (concentration of chromium) for K2Cr2O7. Ten fishes are to be used in each containers and 10 also for the controls. A mortality count were made at the end of every 24 hour period. The criteria for mortality were cessation of gill movement and failure to response to mechanical stimulus.

7. The test would run for 96 hrs. It is advisable from time to time to get the pH and dissolved oxygen level to see any deviation from the control set-up.

9. The data obtained was analyzed using the Completely Randomized Design (CRD) to determine if there is sufficient evidence that with an increase in temperature there is an increased toxicity of petroleum and K2Cr2O7. Thus an Analysis of Variance Table (ANOVA) was constructed for this one way test.

10. The fish were studied for macroscopic and histological examination of the gills. The external features such as the skin, fins etc. were examined. Three fishes per concentration was sampled. All dead and morbid fishes were dissected and proceesed for histopathological changes at the Philippine General Hospital in Manila and National Science and Research Institue in Diliman, Quezon City respectively.

Histopathological Bioassay:

The gills are fixed in 2.5% glutaradehyde in .1 M buffer

(pH 7.2) at 4 degrees centigrade for one hour (see Appendix B for all histological preparations). After overnight washing in phosphate buffer, tissues were postfixed in 1.0% osmic acid in .1 M phosphate buffer (pH 7.2) for one hour at 4 degrees centigrade. Tissues are then washed three times with phosphatebuffer at 10 mins. interval , dehydrated at increasing grades of acetone, embeded in araldite and polymerize for five days at 60 degrees centigrade. Tissues are sectioned at .5 um on an ultrotome with a glass Knife. Sections were stained with methylene blue and counterstained with eosin. Microscopic examinations are done using a light microscope.

11. The time needed for the experiment and the needed expenses are given in Appendix C.

RESULTS

A sublethal concentration of petroleum (1 ppm) & potassium dichromate (130 ppm) was found toxic in S. vulcani (previously indentified as T. nilotica) provided an increase in temperature is administered in its environment. Table 2 showed that for every six S. vulcani placed in a jar, an increase in the no. of deaths & symbol subsequent % death corresponds to an increase in temperature. Sublethal concentration of petroleum acquired a % death greater than 100% for temperature ranging from 30oC-38oC. On the otherhand, the

sublethal concentration of K2Cr207 acquired a % death greater than 100% for temperature ranging from 32oC-38oC. Table 1 depicts an increase in toxicity for both pollutants with regards to time of exposure. This was especially seen in temperatures ranging from 32oC-38oC. Table 1 depicts and increae in toxicity for both pollutants with regards to time of exposure. This was especially seen in temperatures ranging from 32oC-38oC for petroleum & 300oC-38oC for KrCr207 & petroleum would be least toxic at temperature of 18oC-24oC for any period of exposure.

Petroleum at 1 ppm was found to cause a great degree of lethality at lower temp. (28oC) than 130 ppm of K2Cr207. Table 2 proved that at 38oC threshold sublethal concentration of petroleum would increase 13 times a 100% kill. It is noted also that in the case of the 38oC & a 96 hr. exposure, a greater degree of toxicity is attributed to petroleum.

K2Cr207 at 130 ppm was seen to increae in the no. of total deaths 6 times a 100% kil. A 60% kill is at 30oC & begins to climb until threshold temperature of 38oC. Like petroleum, K2Cr207 increases toxicity as a rise in temp. & hr. of exposure is present.

A statistical test (t-test) in Appendix C, revealed that the null hypothesis was rejected. The null hypothesis stated that there is an equality bet. the mean of those killed with a rise on temp. & those without a rise given the sublethal amount of petroleum & K2Cr207. Due to the rejected null hypothesis, an interpretation was stated for both pollutants. It was found out

that there was sufficient that an increase in H20 temperature would increase the no. of deaths of S. vulcani thriving in a sublethal concentration of petroleum (1 ppm) and sublethal concentration of potassium dichromate (130 ppm). The statistical hypothesis was constructed using a .10 confidence interval.

There were some noticeable changes in the fish behavior & morphology in the different pollutants at different temperatures. Although mortalities came at 26oC, in petroleum a weakening of the fish was seen coupled by disoriented movement & suddening blackening of muscles. Although proper coloration wa evident, gill movement became slower and fins were not affected at 96 hr. exposure. In K2Cr207 at 26oC, there was a sudden black discoloration of the muscles after 96 hrs. Increasing the temp. & exposure hour, until the threshold temp., the organism lost its normal straited coloration, lost of weight, increase in the ventilation rate, excessive mucus production, coughing & fin deformation in petroleum. Reduced respiration, reduced feeding rate, loss of weight, weakening, gills were damaged, loss of body balance & excessive mucus production coupled with coughing & formation of a whitish substance around its body esp. the gills were evident in K2Cr207 contaminated water. Both pollutants provided the fish exposed with a smelling stench which can be indicative of change also in its taste. The final acute responses were frenzied activity, followed by death for both pollutants.

The juvenile fishes of S. vulcani (mean weight 7.0 grms with an S.D. = 0.7 grms) were experimented in the vat as seen in Plate no. 1. The temperature was increased using an electric water heater. The data tables 1 and 2 depicted a starting range

of 18oC since the S. vulcani can't tolerate temperature less than this. On the otherhand, the stock fishes were first acclimated at a pool as shown in Plate no. 2. Six fishes only were placed in the fish bowl to minimize during each experiment to minimize the lowering of the dissolved oxygen (DO) and provide the fish with enough space. Plates 3 to 5 shows the normal, petroleum-treated and potassium dichromate-treated fishes respectively under normal conditions of 25oC. Morbid fishes were examined physically and some used for histological examinations.

Plates 6 to 8 depicts the appearance of the necrotic fishes at the end of 24 hrs. at the temperature of 38oC. Plates 9- 11 depicts the morbid fishes at the end of 96 hrs. The upper range of temperature is given emphasis since at this temperature greater morphological and histological damages are seen. It is said that under normal temperature range of 26-28oC, S. vulcani, in sublethal concentration of petroleum and potassium dichromate, exhibits striking changes physically at 96 hrs. of exposure. Plate 4 shows that the fish is in respiratory stress depicted by the blackening of its muscles coupled by rapid breathing. Upon tactile stimulation, the fish responded with weak swimming away from the source of stimulation. This is different in the normal temperature treated fishes (Plate 3) and even in the stock fishes were stimulation is responded by a rapid swimming away from the source. In the potassium dichromte-treated fishes (Plate 4) the same respose of the fish is seen just like in (Plate 5). Bulging eyes, dark pigmentation of the skin and slow gill movement

represents that the fish also has difficulty breathing. A darker pigmentation of the skin results from aggregation of the melanosomes which is brought about by an increase in the pH of the water which is traceable to the Tilapias rapid breathing rate. Petroleum and potassium dichromate diminishes the peripheral store of catecholamine transmitter of the fish which results in the ineffectiveness of sympathetic melanin aggregating stimulation. With an increase in temperature, melanin aggregation of potassium is increased. Thus, like in the trout which was studied by Robertson, S. vulcani increase in temparature induces melanin aggregations of the melanophores.

Increasing the period of exposure at the same time increasing the temperature aggrevates the lethality. The morbid fishes seen in Plates 6 to 8 clearly emphasizes that at 38oC S. vulcani suffer from the effects of thermal stress. Gill ventillation is hampered prompting and increased exhaustion of the muscles. Fishes supply the other cells of their bodies by nutrients through increase protien catabolism and faster distribution of blood prompting ingcrease in reflex bradycardia. Plate 7 and 8 proves this point since the appearance of these fishes appear pale. Although, their pigmentation appear darker esp. in the upper part) than fish grown in normal waters, it is important to note that there are presence of particles suspended in their scales especially in the region near the operculum. Noteworthy also is the frail body structure of these treated

fishes. In regards to the suspended particles present, Plate 7 abounds these particles . Important also is the more slimy feel of the petroleum and potassium dichromate-treated fishes as to the normal water - treated ones.

Eventhough the temperature of the liquid inside the tanks were at 38oC, some fishes were able to survive for 72 hrs. When the fish died , an increase in the slimy feel is noted. Like those who have died in 24 hrs, the 96-hr fish has a darker pigmentation in the experimental test fish (Plates 10 and 11). The deterioration of the digestive system is clearly seen. Olfactory lessions can be observed especially Plate 11. Particles suspended in the fish (esp. in Plate 10 remains the same. Deterioration of the eye and muscles is noteworthy.

Isolating the muscle tissuse of those in Plate 9 to 11 and staining it with methelyne blue and counterstaining it with eosine, would show the lacerations and the impregnation of the some particles believed to be the pollutants. Plate 10 clearly depicts these particles impregnated in the muscle tissues. Taking into account the longitudinal section of the gill filaments of these fishes, the most noteworthy anomaly in the structure is the presence of excess mucus at the tip of the mucuos cells. These execessive secretion of mucus can be found predominantly in the treated fishes. In terms of the degree of mucus production, petroleum > potassium dichromate > normal is the observed order. This can be a good explantion in the slime feeling of these fishes. Noteworthy also is the excess of erthrocytes in the

lamellar capillaries of the treated fishes. In terms of the number of pilaster cells present, normal > potassium dichromate petroleum is the order.

DISCUSSION

The results of the experiments clearly show that an increase in temperature would increase the toxicity of petroleum & potassium dichromate ($K_2Cr_2O_7$) on tilapia S. vulcani. Statistical & histological studies reveal that given a sublethal concentrations of these two pollutants, increasing the temperature of tanks shows an increased in the mortalities of tilapia in 96 hrs. of exposure. Other factors like pH was of significance since S. vulcani's pH range of 7.0 to 7.2 seemed to be less compared to the pH present in the petroleum-treated fishes and greater compared to the $K_2Cr_2O_7$-treated fishes. But it should be noted that eventhough the pH shot up above the pH range of 7.0 to 7.2, S. vulcani show a great degree of survival in tank placed at their optimum temperature even in the presence of the 2 pollutants. But it should be noted that addition of metals like Ag, Cr, Cu, Ni & Zn increases the pH which can depress growth due to cellular destruction. Dissolved oxygen (DO) may also play a role since the more organism in the tank, the less supply of dissolved oxygen & increasing temperatures decrease oxygen sollubility. But in the experiment, only 6 fishes were placed in a tank to reduce mortality due to the effect of DO.

Petroleum even in sublethal concentration is toxic to tilapia S. vulcani in a temp. range of 30oC-38oC. This temperature range iss considered optimum for S. vulcani under normal conditions (14oC-42oC). But it seems like with an addition of petroleum this optimum temperature range becomes lesser. It can also be noted that the fish Tilapia mossambica tolerates 10,000 ppm of diesel or kerosene or gear oil or mobil for 96 hrs. under its optimal temp. (Saha 1983) but with an increasing temp. the story becomes different.

Petroleum toxicity to tilapia S. vulcani stems for the toxicity of its component which increases with a temp. increase. Petroleum hydrocarbon tolerance affects the oxidoreductase system of S. vulcani. The LDH (lactate dehydrogenase) & the suceinate dehydrogenase (SDH) activity in the gills, liver and muscle tissue of exposed and control fishes are affected. LHD activity levels are decreased and SDH levels are increased on tolerance exposure (Ravindan, 1988). LHD is an isoenzyme which catalyzes the transfer of 2 electrons & one H+ from lactate to NAD+ converting 1-lactate to pyrimate. Pyruvate is an essential metabolite for ATP production during the Kreb's cycle. A decrease in this enzyme activity would affect the functions of the gills, liver and muscle tissues. SDH in an isoenzyme which oxidizes succinate to fumarate. It occurs as a ferroflavoprotein which is specific for the transformation of succinate and is inhibited by malonate . Increasing this would be beneficial since electrons &

ATP would be formed ep. during cellular respiration . But coupled with an increase in temperature, protein causes an inactivation of this enzymes (Carins, 1975). An increase in temperature causes alterations in cellular enzymes and membrane lipids. Hellbrum thought that liberation of calcium from the external cytoplasm of some cells in response to heat triggered the release

Toluene also has an effect in the different aminotranferases in the different tissues of tilapia. Activities of both enzymes (aspartate aminotransferase or AAT [EC 2.6.1.1.]) & alanine aminotransferase or AlAT (EC 2.6.1.2.)] in the liver, brain & muscles were elevated (Dange, 1980). The elevated aminotransferase activities indicate increased protein metabolism which was evident in the reduction in soluble protein & free amino acids contents in the tissues of toluene-treated fish. These change can be related with paved carbohydrate metabolism in the stresed fish. The change may also be due to the stressed induce in the circulating levels of the glucocorticoid hormone, cortisol (Dange, 1980). Thus, fih movement, heartbeat, ATP production, etc. are greatly impaired.

Other components of petroleum are n-heptane & n-helane which are toxic to Tilapia mossambica at concentration greater than 1 ppm at pH 7.1 & temp. equal or greater than 27.8oC. Symptoms of exposed fish included damaged gills,respiratory distress, loss of body balance, and deposition of mucus on the gills. Napthalene effects were previously discussed.

It seems that the gills & muscles are the tissuess which are severely attached by petroleum at increasing temp. It is believed that exposure to petroleum induce histopathological effects. The gills tend to harbor parasites but under normal conditions causes no or little harm. With addition of petroleum, these organism increases. Trichodinid ciliates are mainly ectoparasitic protozoans on the gills of fishes produce an increased prevalence & intensity of parasitism (Khan, 1990). Thi may contribute to the occluison of the gills, gill epithelial sloughing, decrease gill mucus cell concentration, etc. which is present in petroleum stressed fishe. Mentioned earlier, petroleum causes an increased protein metabolism due to increase activity of AAt & AlAT compensating for the impairment of carbohydrate metabolism. It should be noted that with an increase in temperature, enzymatic activity is affected. The same effect in protein effect can be attributed to the muscles. This is coupled by the fact that petroleum under increasing temperature increases in abdominal muscle fiber degeneration coupled by an increase in the frequency of dilation of Bowman's space with glomerular hypertrophy (Haensly, 1982).

Other organs affected also include the liver and spleen. Liver lesions in the form of an increase in the rough endoplasmic reticulum which results in a high lipid vacuoles resulting in swelling, liver enlargement & producingt a yellow while

coloration of the liver. splenic discolorations & lessions are observed due to the action of no. 2 fuel oil in an Exxon Valdez oil spill (Khan, 1990).

Chromium toxicity is also increased by an increasing temperature to the tilapia S. vulcani. Unlike petroleum, the lethal range for chromium is at 32oC-38oC, two degrees higher than petroleum. Also unlike petroleum, sublethal concentration of potassium dichromate in increasing temperature is largely dependent on pH and 50% survival at 28oC to 30oC compared to petroleum's 26oC to 28oC range. But like petroleum, the gill and muscles are affected so well as other tissues like the liver.

Hexavalent state of chromium is more assimilated by S. vulcani therefore more toxic than the trivalent form. At physiological pH the relationship between chromate and dichromate ion proceeds as follows:

$$Cr2O7 + H2O = 2\ H\ Cr\ O4 -$$

This relationship depends upon pH, dilution and heat. It can depress growth of algae Salenastrum Capricornutum Printz and detected in trace quantities but apparently place no essential role in the physiology of plants or even animals. This depression and growth can be credited to the high toxicity of this metal due to cells killed (Michnowicz et al, 1984). Sublethal effects of heavy metal (zinc, cadmium, mercury, copper, chromium, arsenic, iron & manganese) among fresh H2O aquatic animals. The study proved that copper & mercury have the highest

toxicity; chromium (hexavalent) & manganese (divalent) have the lowest toxicity. The toxicity of potassium dichromate to Daphoria is about 2,700 times to that to fish. It also mentioned the influence of pH and a decrease of hardness of H20 increases the toxicity of these metals. It stressed the fact that Tilapia is the most resistant to heavy metals among the tested fishes (Chen, 1980).

Respiratory and feeding rates of exposed T. mossambica were reduced at pH 6.0 to 7.0. Production of fish, fecundity (at pH 7.0 & 8.0) & breeding were reduced in chromium pollution. Growth & reproduction were hampered by sublethal mixture. Mixture of sublethal concentrations of component metals (mercury-chromium-cadmium) became lethal to T. mossambica & Cyclops Viridis & the worm Branchiura sowerbyi (Kaviraj, 1983). These are some immediate effects of sublethal concentrations of potassium dichromate.

An increase in temperature would affect the gills of S. vulcani. Depression of respiration, a common feature in acute poisoning, may be reflected by changes in the tissues of the respiratory organ. The gills amount and functional state of mucus glands, thickness & cellularity of respiratory epithelium is clearly affected (Hinton et al, 1983). Histopathological/ultrastructural changes in various tissues of grass shrimp (Palaemonetes pugio) exposed to hexavalent chromium did not induce marked changes in the gills but caused invasive

melanized cuticular lessions. Chromium also caused apparent labyrinth hypoactivity ion the antennal glands (Rao, 1984). In studies in the gills of rainbow trout (Salmo gairdneri), the transfer of chromium is directly coupled with the transfer of oxygen from the external solution to the internal perfusion medium. It also showed that chromium transfer was significantly more effective at pH 6.5 than that of pH 8.1. It also stressed that chromium accumulated by the gill tissue inreased at lower pH (Van der Putte, 1983). In S. vulcani, the decrease in pH which results due to an increase in time (96 hrs.) of exposure took a greater degree of mortality especially for those which were placed at higher temperature. Experiments also showed that sublethal concentration of chromium alone produces no change in the activities in the lactate dehydrogenase (LDH), pyrivate dehydrogenase (PDH), succinate dehydrogenase (SDH) in the gills, liver, muscle, kidney & brain (Sastry, 1982). Increasing the temp., would affect these enzymes prompting it to decrease in activity & hamper gill movement resulting to the death of a fresh H2O teleost like the Channa punctatus or even a tilapia. Mortalities due to gill accumulation of a flourescent whitening agent known as Blankophor is seen in goldfish Carasius auratus independent of dose & temperature of potassium dichromate (Riva, 1981). The experiment clearly presents that the presence of Blankophor, not only in the gills but other tissues especially the skin, was evident in S. vulcani under any temperature. This finding also supports the previous one in identifying that gills

of moribund fish has a greater degree of chromium compared to living fishes under the same treatment.

Due to the denaturation of certain enzymes or decreased activity, chromium at higher temperature produces a lesser amount of muscle glycogen in Channa punctatus (Gosh, 1985). This results to the weakening of the red muscle which is interdigitated with the white muscle and is considered the origin of propulsive force for fish and mammals. Together with the white muscle, it powers the high speed locomotion of fishes. Red muscle undergoes aerobic metabolism while the white one undergoes anaerobic metabolism. Due to the decrease in muscle glycogen S. vulcani shows a stagnant, less active movement in chromium waters even under normal temperature. With a subsequent increase in temperature, the activity of movement increases but as the temperature goes beyond optimal, enzymes which form ATP from the hydrolysis of APP + Pi are destroyed or lessened in activity. This is coupled by the fact that temperature beyond optimal causes cellular and epithelial damages to the muscle. This results to a high increase of NH4 + excretion which lowers the pH of the tank prompting an increase in chromium toxicity of lethal levels. The study of Kitty in 1972 noted that the tilapia excreted excessive amount of NH4+ compared to the amount of oxygen consumed suggesting a utilization of amino acids as sole carbon and energy source. This free amino acid increase in order of: muscle liver > brain > gill > kidney. This is due to enhanced proteolysis coupled by stepped up protein synthesis.

Glutomate dehydration was inhibited in all tissues in the order: gill muscle brain liver kidney which reveal the reduction of decrimination in exposed fish (Gosh, 1985). This compensatory adjustment increases the blood glucose and lactic levels prompting increased activity of the white muscle which acts as a powerful but temporarily limited energy source for vigorous movement. As long as tissue damage does not occur muscle glycogen would inbcrease and would also increase muscle LDH and PPH activities resulting to an increased rate of glycolysis as long as the enzymes are not denatured. This could be a reason why Tilapias are more resistant to chromium toxicity compared with other organisms.

Petroleum, potassium dichromate or any other pollutant can affect S. vulcani either directly, indirectly or inducely. A direct effect is caused only by the direct action of the toxic agent on fish. Indirect ones are those caused by an action of toxic agent on something than fish which in turn causes an effect on fish (ex. effect of chromium on pH). Induced effects are those brought about by a direct action of the toxic agent on the fish but which can only occur in the presence of another agent (Stephan et at, 1983). The effect of temperature is an example of an induced effect.

A temperature increase potentiates the effect of sublethal dose of these pollutants. Temperatures alone may be a lethal factor but in S. vulcani, optimal temperature is reduced

with a presence of these pollutants. Subjecting the Tilapia beyond its temperature zone resistance results in thermal death. But subjecting the same Tilapias to their optimal temperature with sublethal dose of pollutant results also to the same death.

This kind of death shows many manifestations. Tissue anoxia in which the toxicant increase its lethality by rendering its components more active. All aquatic organisms would exhibit failure of osmoregulation, alteration in cellular enzymes and membrane lipids, protein denaturation, decreased metabolic activity, decrease in tissue H2O content etc. subsequently affects fish behavior, movement and other physiological functions. Changes in lipid composition of cellular membranes are affected with an increase in temperature; this action affects the fluidity of the membrane which results in a morphological damage of the tissue at the same time affect the fluidity of the mitochondrial membrane which results in the change in the rate of oxidative metabolism. This also results to a decrease in muscle contraction & increased muscle exhaustion. Increase in temperature prompts increases the pathway to glycogen and the HMP shunt but the destruction or denaturation of decreases glycogen metabolism. This prompts certain fishes like the S. vulcani to seek protein as a source of energy which could either manifest in a decreased swimming (especially 30oC-38oC) & increase in iron uptake (70%) in erythocytes increasing heartbeat and subsequently increasing oxygen demand in respiration. Death results if the respiratory organ is blocked or destroyed by certain pollutants &

mucus. Rapid swimming drops with a rhythmic block of discharge pattern of respiratory neurons in the medulla which relates closely in time to the development of severe hypoxia in the brain of the tilapia as a result of prior loss of good cardio-ventilatory efficiency. Hugh & Roberts demonstrate that this cardio-ventilatory efficiency can induce reflex bradycardia in bony fishes.

An increase in temperature increases the diffusion of particles suspended in water. This is due to the fact that the particles suspended move faster. Since the pollutants studied are generally toxic at even sublethal levels, the increased diffussion of its particles on S. vulcani prompts a lesser time of contact to the fish and greater efficiency in decreasing the fish physiologic activities. Death may come at an alarming rate. The increase in diffusion of the pollutants to the fish affects the tissue H20 content. A decrease in H20 content inside the fish tissues brought by an increase in concentration of solute particles (pollutants) in the external solution and by osmosis deprive the tissue with an important metabolite (H20) in metabolism (ex. cellular respiration). This hydration of tissues may have repercussions in cellular integrity at the same time decrease physiological efficiency. The diffusion of particles affects the osmotic and ionic balance of gills. At high temperature passive ion flux is excessive compared to actie transport prompting the effective entry of the pollutants for

gill occlusion. This affects the fish respiration which can also alter the O2 requirements and CO2 production which affect also the acid-base balance in the test tank. Therefore gill ventillation, muscle activity, & cardiac output is affected.

It is recommended that farmers following the "palay-isdaan" concept of fish farming mut be aware of the ill-effects of these pollutant and find ways to detect their presence. For petroleum there are certain enzyme which could degrade its lethal components and for potassium dichromate there is a colorimetric method to detect the hexavalent chromium. The experiment failed to recommend what are the "safe" level of petroleum and potassium dichromate since both were considered toxic even in sublethal levels and were used in conjuction with temperature. It is wise for farmers to determine the optimal temperature range of their tilapia and protect their cultures for petroleum spills or contamination of hexavalent chromium in the form of fertilizers. Acclimatization of tilapia is essential to its survival thus farmers must see to it that before subjecting Tilapias to high temperature acclimatization to a lower one and gradually increasing the temperature is essential. These processes could assure their Tilapias to be reproductive, large and resistant to any disease. This would make their S. vulcani devoid of any foul smell and have qualities worth of competing in foreign market.

S. vulcani were placed in tanks with normal water, petroleum-treated water (1ppm) and 130 ppm K2Cr2O7-treated water. These concentrations of pollutants were considered to be sublethal. Experimental tanks containing the same treatments of water were assembled. These tanks were immeressed in an unconcealed vat filled halfway with water. Six S. vulcani were placed in these tanks after undergoing acclimatization in a pool. The temperature of these experimental tanks were increased using an electric heater from 26-38oC. The electric heater was placed on the water of the unconcealed vat and taking into consideration the time of heat conduction to the experimental tanks, the desired time of heat application was known. It was found out that for every hour, the heat dissipated into the experimental tank was the same heat first applied to the outside water. It was logical that in order for the water in the experimental tank to be constant, temperature should be increase up to the desired temperature every hour. This system of water-bathing the experimental tanks substituted the set-up wherein continous supply of heated water is pumped into the experimental tanks to achieve a constant temperature within the tanks. The experimental tanks were decreased in temperature using the same method but instead of an electric heater, cubes of ice were used. The temperature was lowered to 18oC only since the tilapia was

tolerant to this temperature. Temperature lower than this would kill the tilapia instantly. This two methods seemed to be crude but is effective especially when the temperatures are frequently monitored.

The tilapias were then subjected to temperatures of 18o-38oC for 24,48,72 and 96 hrs. Since 6 tilapias per experimental tanks were used, morbid fishes were replaced by fishes from the acclimatization pool (with temp.= 28oC) every 24 hrs. It was found out that at the end of the 96 hr. period., the fishes in 1 ppm petroleum increased in the percentage of death 13 times the a 100% kill. It also showed that at 38oC, 78 were killed compared to the 36 kills of 130 ppm of K2Cr2O7 at the 96 hr. period. At 130 ppm K2Cr2O7, a 96 hr period-38oC water temp. 6 times a 100% kill is seen. It can be noted also that at a range of 18-24oC, no deaths can be existent. It was also observed that for every increase in temp., there was an increase in the no. of deaths.

Death was counted by using the cessation of gills, failure to respond to stimuli and inverted posture without swimming as the criteria. A physical examination of the morbid fish would reveal that under sublethal concentrations of petroleum and K2Cr2O7, these fishes have a darker skin pigmentation compared to the normal fish at higher temperatures. It is also noted that there are lacerations and destruction of the muscles, buccal cavity, eyes and GIT of the pollutant-treated fish compared to the fish grown at normal waters. This condition

is aggrevated more if these fishes were placed at higher temp. An increase in the slimy feel of the morbid fish grown in pollutant treated waters reveal that secretion of mucus is greater. This was clearly seen in the petroleum treated fish. It was evident that this slimy feel increased due as an increase in the temperature of the water. In fishes grown in K2Cr2O7 waters, a whitish substance known as a blankophor is seen in the fish's scales, gills and surrounding body parts (external). In general, at increasing temeperature whether grown in normal or pollutant-treated waters, the fish body becomes frail which gives a clue to its slow growth.

Using a statistical test (t-test), an increase in temperature would show an increase in death of tilapia S. vulcani in sublethal concentrations of petroleum and K2Cr2O7 is the arrived conclusion. Histological bioessays also supports these findings since by examining the muscles and gill filaments, the residues of these pollutants cling to these tissues producing complications in thier physiological functions. Enzyme deactivation and denturation are the primary effects of an increase in temperature prompting an abnormal physiological fuctions of these organs. It should be remembered that the destruction of the GIT also gives a support to this increasing death rate.

An increase in the temperature increases the toxicity of petroleum and potassium dichromate in S. vulcani even in sublethal levels. The greater the time of exposure, the greater

is the mortality. The results clearly showed that with an increase in temperature, the effects of sublethal dose of the two pollutants are aggrevated. Destruction of the cellular constituents, denturation or incativity of the vital enzymes, alterations in the physiological processes, alteration in behaviour, occurence of thermal death etc. are some of its manifestations. Therefore, temperature, being a lethal factor itself , induces an increase in toxicity of sublethal concentrations of petroleum and potassium dichromate when applied to the tilapia S. vulani.

RECOMMENDATIONS

This study is a pioneering study in regards of correlating temperature to the toxicity of petroleum and potassium dichromate to the tilapia S. vulcani. It is feasible to also make some studies regarding the effect of some pollutants other than this two. It must be pointed out that any errors in this study would be attributed to the inconsistency of the temperature maintenance in the experimental tanks due to the inavailability of the materials and due to human error. But essentially this study could pave the way for the exploration of the different effects of the 2 pollutants in terms of their manner and degree of kills to the tilapia.

It is also feasible to study the effects of these pollutant to other tissues of the GIT, liver and etc. It is

therefore recommended that farmers using the "Palay-isdaan" system of tilapia culture must bear in mind the effects of these 2 pollutants to their cultures. Assuming that given the support of the government, studies concerning to the development of a strain of tilapia capable of withstanding lethal concentrations of the 2 pollutants is highly recommended. The findings in this study would be beneficial in determining the criterias geneticists would need in developing the strain. It is also possible to correlate the effect of pH and dissolve oxygen (DO) extensively to pollutants other than petroleum and K2Cr2O7. Lastly, a study to find ways and means to determine the effects of digesting morbid fishes ,exposed to higher temp. and sublethal concentrations of petroleum and K2Cr2O7, to the human body can be of practical endeavor.

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TABLE 1. Data gathered showing the temperature, survival time, average pH for every survival time, number of deaths and the percentage of deaths for every pollutants.

		1 PPM PETROLEUM			130 PPM POTASSIUM DICHROMATE		
TEMPERATURE (°C)	TIME (HRS)	AVERAGE pH	NUMBER OF DEATHS	PERCENTAGE OF DEATHS	AVERAGE pH	NUMBER OF DEATHS	PERCENTAGE OF DEATHS
18 °C	24	8.31	0	0	7.50	0	0
	48	8.27	0	0	7.50	0	0
	72	8.02	0	0	7.50	0	0
	96	8.00	0	0	7.50	0	0
20 °C	24	8.33	0	0	7.51	0	0
	48	8.29	0	0	7.52	0	0
	72	8.04	0	0	7.51	0	0
	96	8.02	0	0	7.50	0	0
22 °C	24	8.29	0	0	7.55	0	0
	48	8.25	0	0	7.54	0	0
	72	8.00	0	0	7.52	0	0
	96	7.98	0	0	7.53	0	0
24 °C	24	8.31	0	0	7.55	0	0
	48	8.28	0	0	7.54	0	0
	72	8.02	0	0	7.53	0	0
	96	8.00	0	0	7.53	0	0
26 °C	24	8.32	1	16.67	7.56	1	16.67
	48	8.30	0	0	7.55	0	0
	72	8.28	0	0	7.55	0	0
	96	8.25	0	0	7.54	0	0
28 °C	24	7.50	1	16.67	7.00	1	16.67
	48	7.43	1	16.67	6.80	1	16.67
	72	7.40	1	16.67	6.80	0	0
	96	7.33	1	16.67	6.80	0	0
30 °C	24	7.10	4	66.67	7.00	1	16.67
	48	7.10	0	0	6.80	0	0
	72	7.08	3	50.00	6.80	0	0
	96	7.06	1	16.67	6.75	4	66.67
32 °C	24	6.95	2	33.33	6.80	1	16.67
	48	6.94	2	33.33	6.75	2	33.33
	72	6.90	4	66.67	6.74	2	33.33
	96	6.89	4	66.67	6.73	3	50.00
34 °C	24	6.80	4	66.67	6.73	1	16.67
	48	6.73	4	66.67	6.70	3	50.00
	72	6.70	4	66.67	6.70	2	33.33
	96	6.65	5	83.33	6.68	3	50.00
36 °C	24	6.50	6	100.00	6.66	3	50.00
	48	6.40	5	83.33	6.63	4	66.67
	72	6.39	5	83.33	6.55	5	83.33
	96	6.36	6	100.00	6.50	6	100.00
38 °C	24	6.25	8	133.33	6.40	6	100.00
	48	6.22	12	200.00	6.39	8	133.33
	72	6.20	24	400.00	6.38	10	166.67
	96	6.10	36	600.00	6.36	12	200.00

TABLE 2. Summary results of the number of deaths and percentage of deaths in the corresponding temperatures for the two pollutants.

	1 PPM PETROLEUM		130 PPM POTASSIUM DICHROMATE	
TEMPERATURE (°C)	TOTAL NUMBER OF DEATHS	PERCENTAGE OF DEATHS	TOTAL NUMBER OF DEATHS	PERCENTAGE OF DEATHS
18- 24 °C	0	0	0	0
26 °C	1	16.67	1	16.67
28 °C	4	66.67	2	33.33
30 °C	8	133.33	4	66.67
32 °C	12	200.00	8	133.33
34 °C	17	283.33	9	150.00
36 °C	22	366.67	18	300.00
38 °C	78	1300.00	36	600.00

APPENDIX A

Analar chemical used to obtain a hardness of 2.

KCL	.02	2.68×10 molar
Na_2SiO_3	.02	1.63×10 molar
$NaHCO_3$	.04	4.76×10 molar
$MgSO_4.7H_2$	.04	1.62×10 molar
$Ca(NO_3)_2$	.03	1.82×10 molar
$CaCO_3$	.01	1.00×10 molar
K_2HPO_4	.01	5.73×10 molar
Fe (ferric citrate)	.0004	

APPENDIX B

HISTOLOGICAL PREPARATIONS

I. Solutions:

1. 2.5% glutaraldehyde

Dilute 2.5 ml of 25% glutaraldehyde with buffer up to 25 ml.

2. Phosphate buffer, pH 7.2

Solution A - 14.196 g sodium phosphate dibasic, dissolved in 1 liter distilled water.

Solution B - 13.609 g potassium phosphate monobasic, dissolved in 1 liter distilled water.

Mix 3 parts A and 1 part B

3. Wash buffer

5% sucrose in phosphate buffer.

4. 1 % OsO4

100 mg OsO4in 9.9 ml. phosphate buffer.

5. Araldite

Araldite M ..... 50 g

Hardener HY964.....40 g

Accelerator DY064 .....2 g

Mix in beaker with automatic stirr it until homogenous(20-25 mins.).

APPENDIX C

Budget for Materials

A. Chemicals

Analar Grade Chemicals	200.00
K2Cr2O7	200.00
Petroleum	80.00
fish feed	50.00
chemicals for hist. prep.	200.00
TOTAL	730.00

B. Experiment Materials

5-gallon Pyrex plastic jars	200.00
aeration tube	50.00
S. vulcani	80.00
TOTAL	330.00

C. Instruments

pH meter

thermometer

GRAND TOTAL 1060.00

* all are in pesos

Time Requirement

A. Experimental Set-up 1 week

B.	Data Collection	2 weeks
C.	Histological Exp.	5 days
D.	Data interpretation	1 week
E.	Typing	1 week
	TOTAL	5 weeks and 5 days

APPENDIX D

A. No. of deaths at 1 ppm petroleum

PERIOD (HRS.)	24	48	72	96	
INCREASED IN TEMP. (26-38oC)	26	24	41	53	
WITH NO INCREASE IN TEMP.	0	0	1	3	
d_1	26	24	40	50	= 136
d_1^2	676	576	1,400	2,500	= 5352

1.) Ho: mean of dead fishes in sublethal concentration of petroleum with increasing temp. is equal to the mean of dead fishes thriving in the same conc. but with no increase in temp.

Hi: mean of dead fishes in sublethal concentration of petroleum with increasing temp. is greater than the mean of dead fishes thriving in the same conc. but with no increase in temp.

2.) Level of confidence = .10

3.) Critical region: t 1.638

degrees of freedom : $v = n - 1$

$v = 4 - 1 = 3$

$$t = \frac{\bar{d} - d_o}{sd \,/\, \text{square root of } n}$$

$\bar{d}$ = summation of d_i/n = 136/4 = 34

$$s_d^2 = \frac{\text{summation of } d_1^2 - (\text{summation of } d_1)^2/n}{n - 1}$$

$$= \frac{5352 - (5352/4)}{4 - 1} = 1338$$

sd = 36.58

t = 1.8589

since 1.8589 > 1.638

4.) Decision : Reject Ho

5.) Interpretation : There is sufficient evidence to support that

water temperature would increase the no. of deaths of Tilapia thriving in a sublethal concentration of petroleum.

B. No. of death in 130 ppm potassium dichromate

PERIOD (HRS.)	24	48	72	96	
INC. IN TEMP. (26-38oC)	14	18	19	28	
WITHOUT INC.	0	0	1	2	
d1	14	18	18	26	= 76
d_1^2	196	324	324	676	= 1520

1.) Ho: mean of deaths of fishes in sublethal concentration of potassium dichromate with an increase in water temp. equals the mean of deaths in tanks with the same conc. but without an increase in temp.

Hi: mean on deaths of fishes in an increased water temp. with sublethal conc. of potassium dichromate is greater to the mean of death in fishes without an increase in temp. but the same conc. of the pollutant.

2.) Level of significance = .10

3.) Critical region : t > 1.638
degrees of freedom = 3

d = 19

square of the standard deviation = 380

standard deviation = 19.49

Computed critical region = 1.949

since 1.949 1.638

4.) Decision: Reject Ho

5.) Interpretation : There is sufficient evidence that an increase in temperature would increase the no. of deaths of Tilapias thriving on a sublethal concentration of potassium dichromate than those thriving in the same concentration but without an increase in temp.

Plate 1. A tank where the experimental fishes were placed. A theremometer was used to the temperature increase.

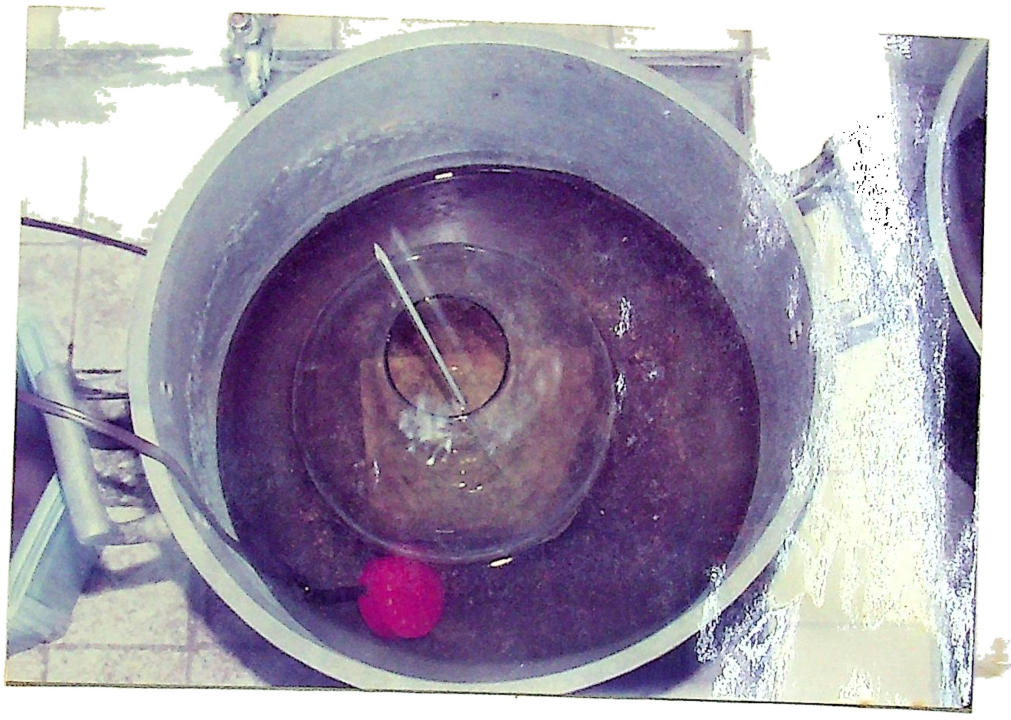


Plate 2. The pool where the stock fishes were confined.

Plate 3. A fish exposed to normal temperature of 26oC in normal water.

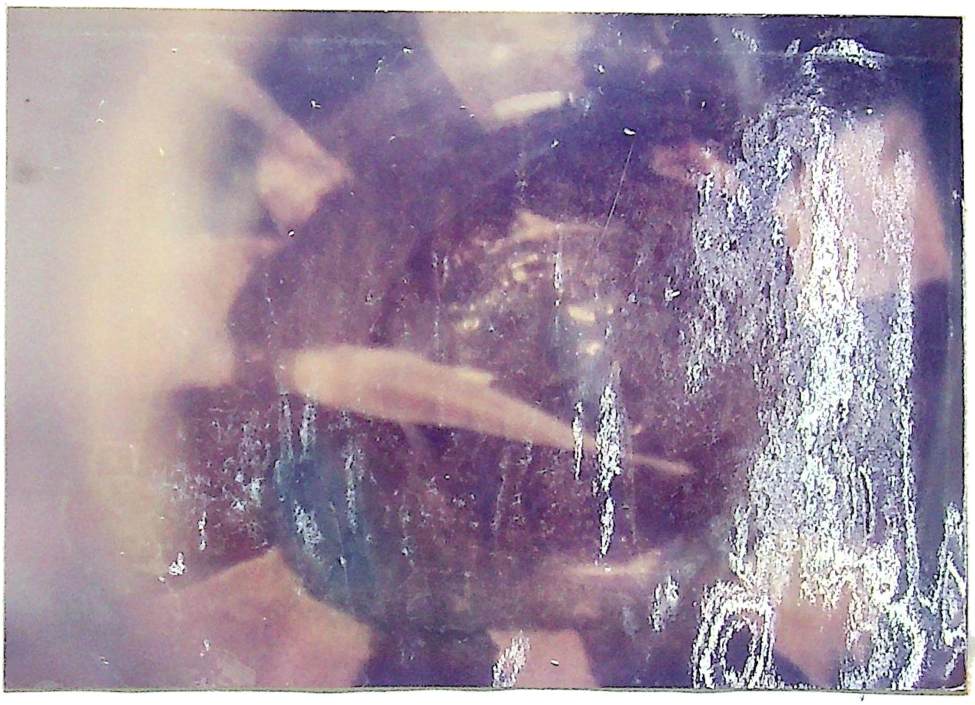


Plate 4. A fish exposed to normal temperature of 26oC in $K_2Cr_2O_7$-treated water.

Plate 5. A fish exposed to normal temperature of 26oC in petroleum-treated water.

Plate 6. Necrotic Fishes that have been subjected to 38oC water temp. for 24 hrs. in normal water.

Plate 7. Necrotic Fishes that have been subjected to 38oC water temp. for 24 hrs. in K2Cr2O7-treated water.

Plate 8. Necrotic Fishes that have been subjected to 38oC water temp. for 24 hrs. in petroleum-treated water.

Plate 9. Necrotic Fishes that have been subjected to 38ôC water temp. for 96 hrs. in normal water.

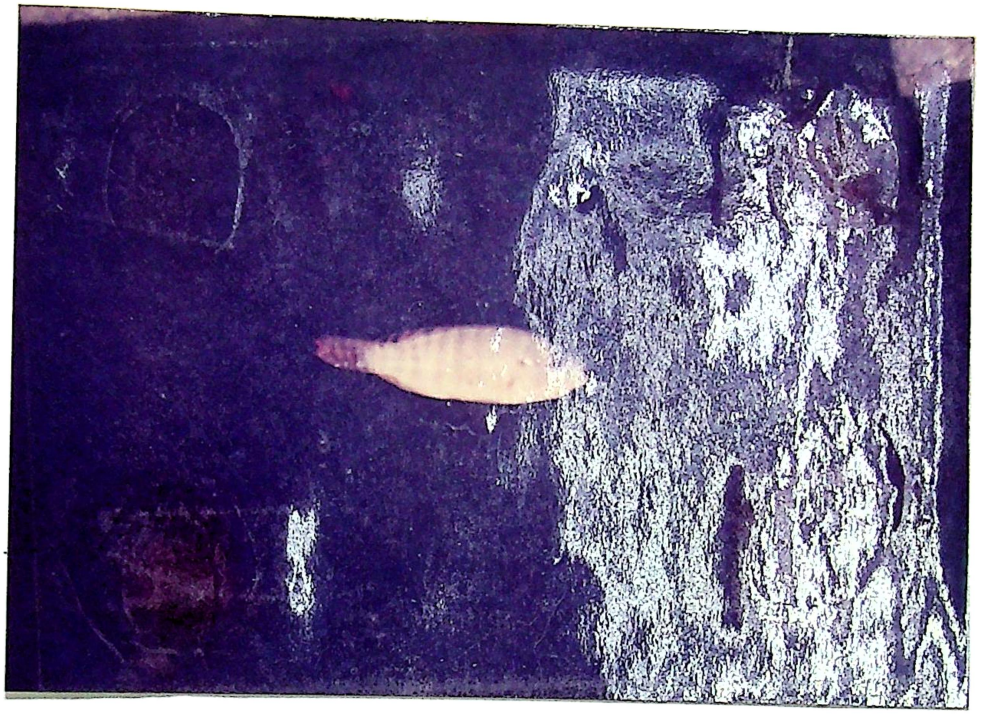


Plate 10. Necrotic fishes subjected to 38oC water temp. for 96 hrs. in K2Cr2O7-treated water.

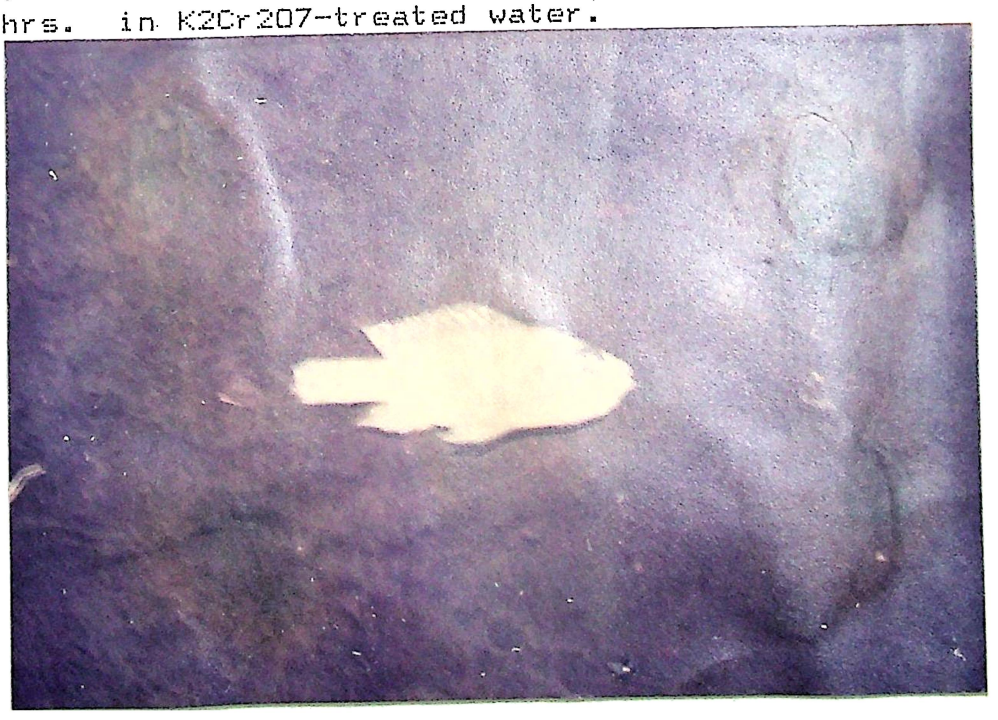


Plate 11. Necrotic fishes subjected to 38oC water temp. for 96 hrs. in petroleum-treated water.

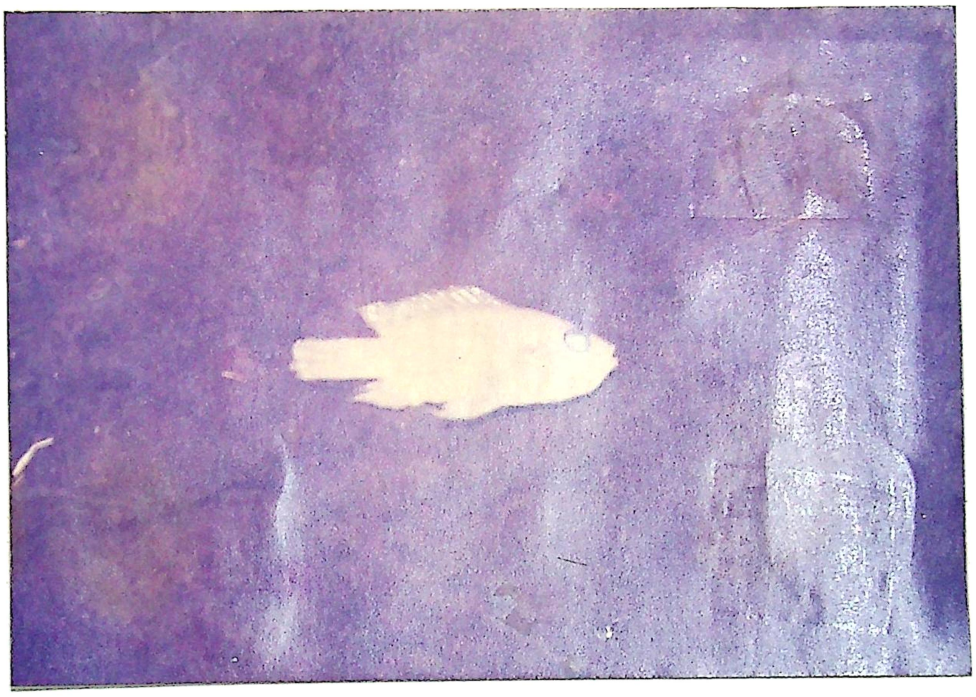


Plate 12. Muscle tissues of fishes exposed for 96 hrs. with temp. of 38oC in normal water. (x 100 mag.)

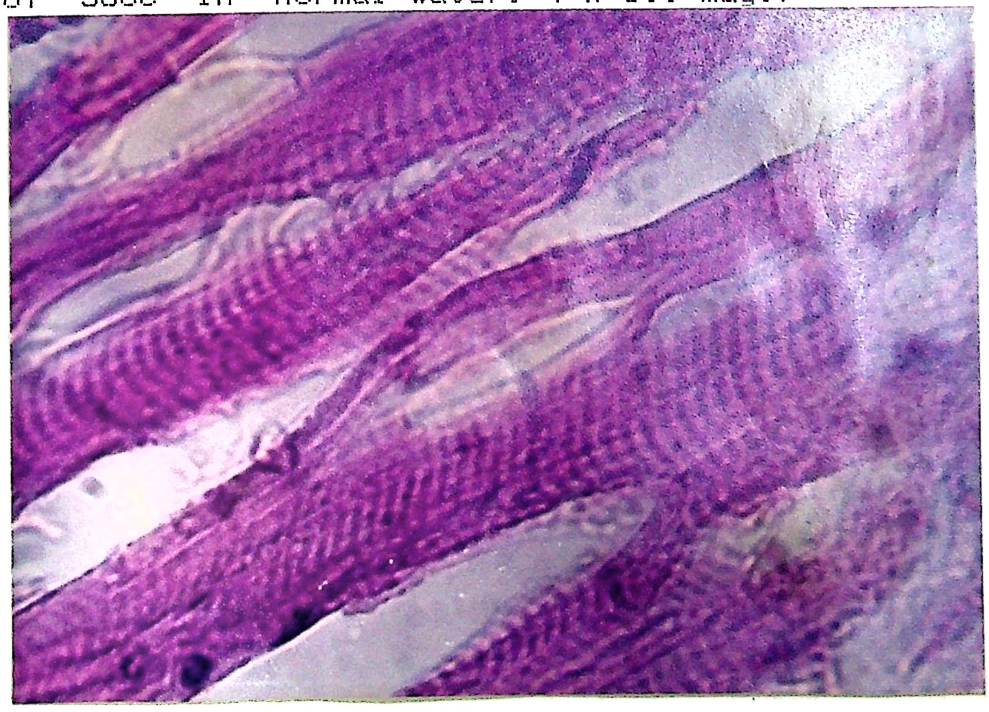


Plate 13. Muscle tissues of fishes exposed for 96 hrs. with temp. of 38oC in K2Cr2O7-treated water. (x 100 mag.)

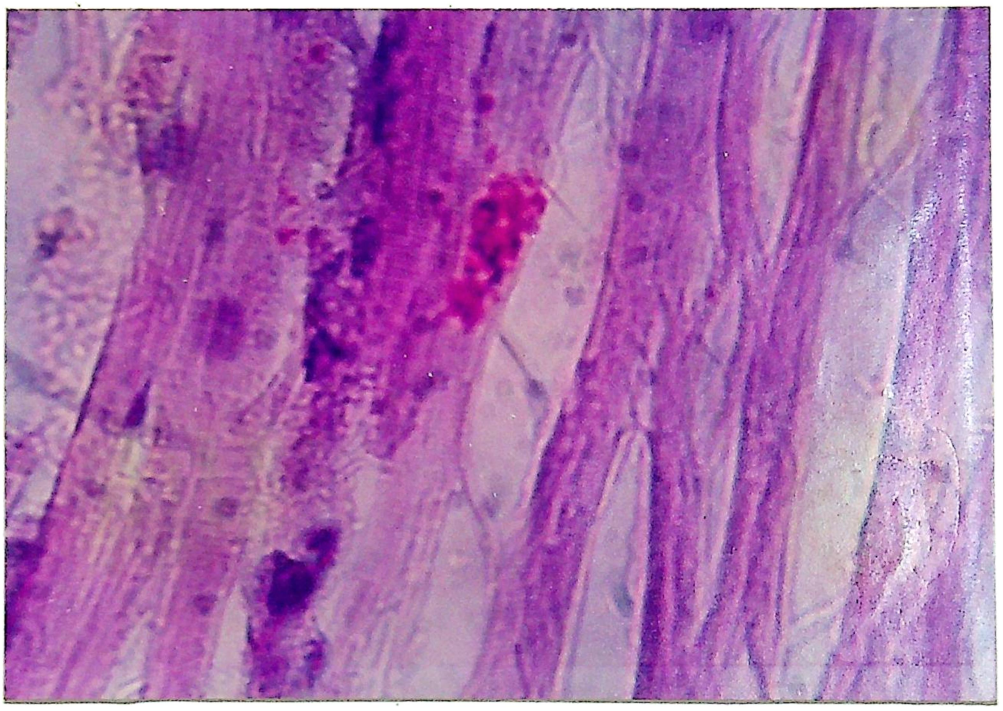


Plate 14. Muscle tissues of fishes exposed for 96 hrs. with temp. of 38oC in petroleum-water. (x 100 mag.)

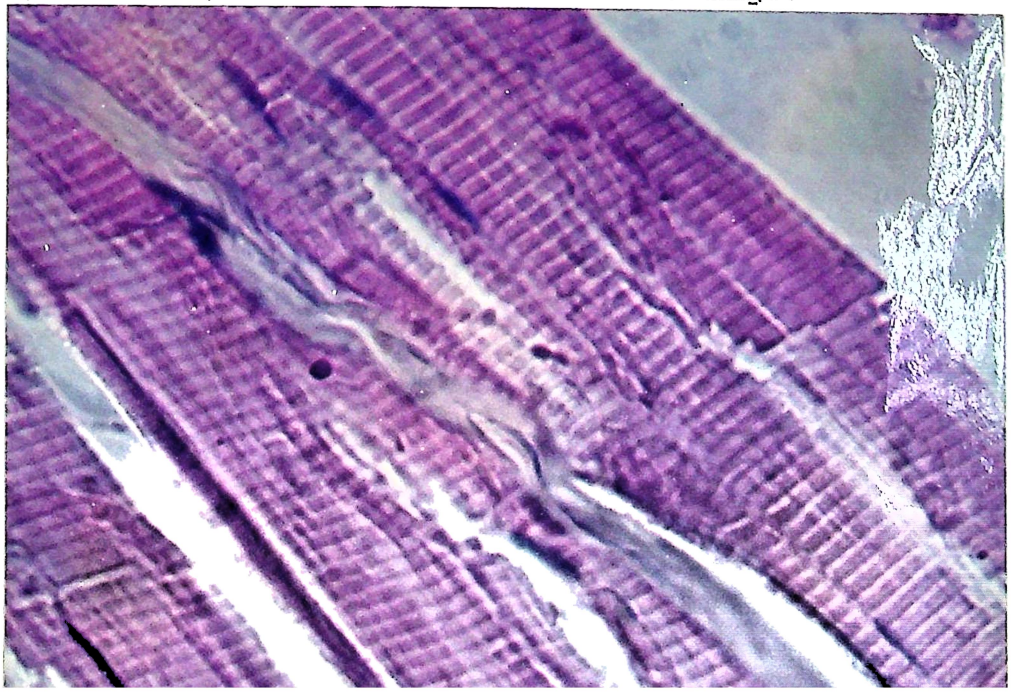


Plate 15. Gill filaments of fishes exposed to 96 hrs. of 38oC in normal waters. (x 100 mag.)

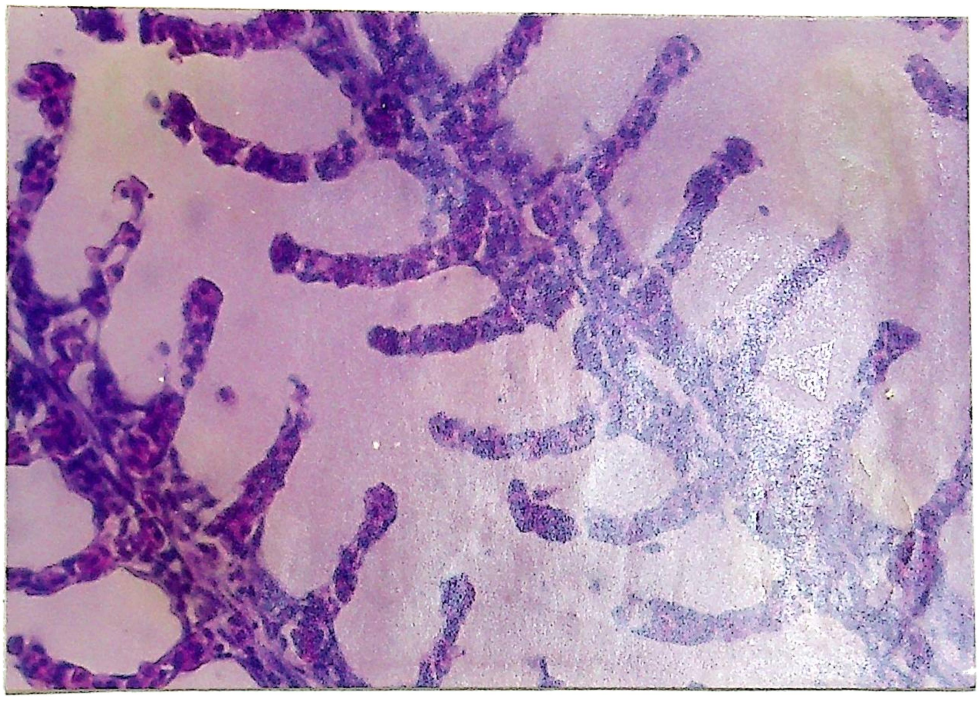


Plate 16. Gill filaments of fishes exposed to 96 hrs. of 38oC in K2Cr2O7-treated waters. (x 100 mag.)

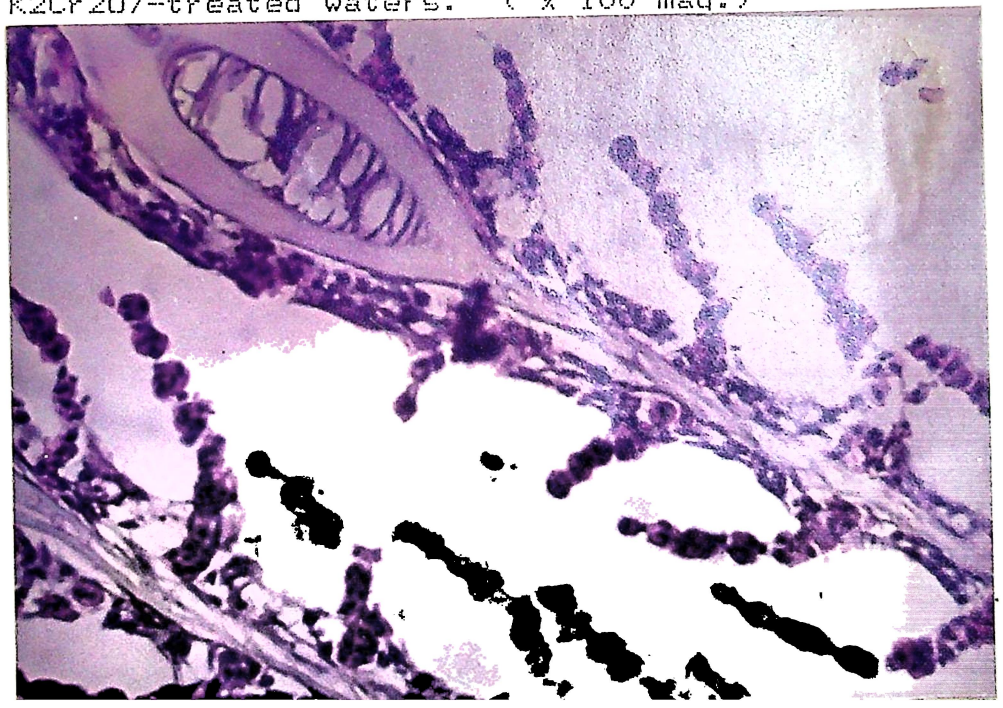
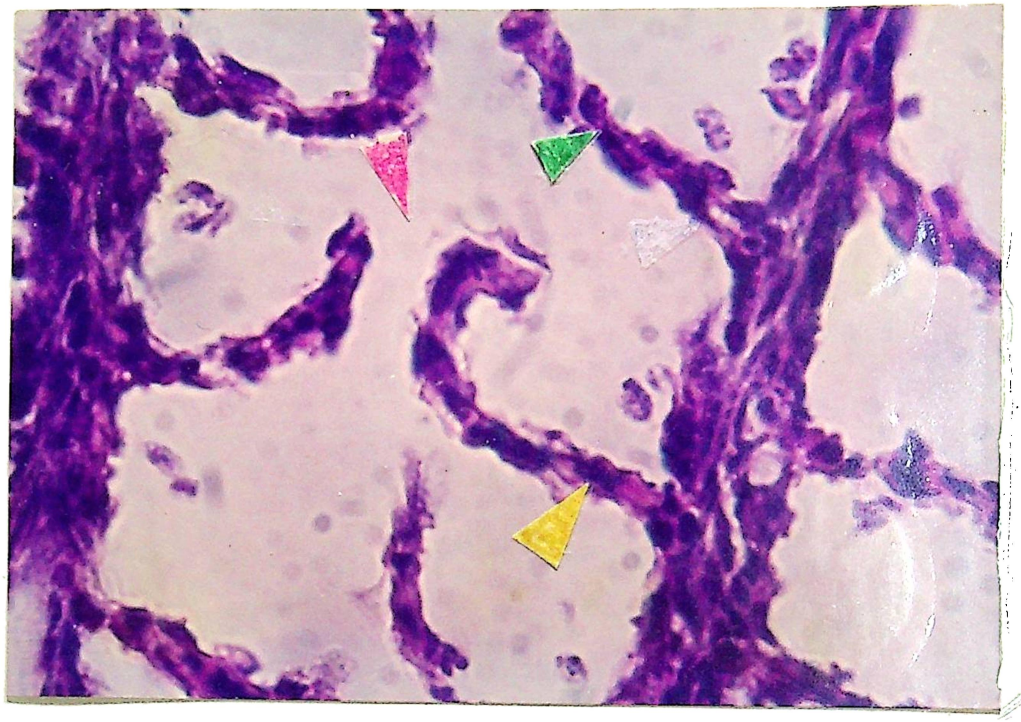


Plate 17. Gill filaments of fishes exposed to 96 hrs. of 38oC in petroleum - treated waters. (x 100 mag.)



Legend:

- pollutant particles.
- muscle fiber nucleus
- lacerations
- mucuos cells
- erythroctes in lamellar capillary
- pilaster cells
- gill lamellum
- mucus