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In Vivo Changes in the Activity of Gill ATPases, Muscle Cell Volume, and Plasma and Cellular Ionic Concentration of the African Catfish, *Clarias gariepinus* Induced by Atrazine or DDT at Different Sublethal Concentrations

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ABSTRACT. The study concerns the effects of the herbicide atrazine and the insecticide p,p'-DDT at different sublethal doses and during various test periods on branchial Na^+, K^+, Mg^{2+}-ATPase activities, ionic composition of the plasma and muscle and the volume of muscular cells in the african catfish *Clarias gariepinus*. The most important significant changes in Na^+, K^+, ATPase activity during exposure to atrazine were represented by decrease at the lower concentration (100 μg/liter) and increase at the higher concentration (500 μg/liter). Exposure to 500 μg/liter DDT produced a short and strong activation of gill Na^+, K^+,- ATPase which was followed by recovery to controls level. While Mg^{2+}-ATPase was activated from exposure hr 72 at both atrazine concentrations, it was inhibited during the whole experimental time in DDT (24 hr). Sodium and potassium concentrations in plasma and muscle cells as well as cell volume were changed during exposure to both chemicals, which might indicate osmoregulation difficulties. The value of the measured responses as an indicator of stress caused by water contamination is discussed.

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Introduction

Osmotic regulation in teleosts is intimately bound to control of ionic concentration as well as cell and body volume (Assem and Hanke, 1979, Abo Hegab and Hanke, 1981, 1986). Branchial adenosine triphosphatase (ATPase) enzymes play an important role in facilitating the transfer of electrolytes across the epithelium (Jampol and Epstein, 1970, Zaugg and Mclain, 1970). Several authors have noted effects of exposure to the herbicide atrazine or to the chlorinated insecticides notably p,p'-DDT on gill ATPase activities in vivo in fish (Gluth and Hanke, 1983; Assem, 1984 and others). The lipophilic xenobiotics may bind to enzyme system in a number of ways dependent upon the molecular structure and availability of functional groups of the enzyme (Watson and Beamish, 1981) their binding on active sites or remote locations on the enzyme molecule may result in the inhibition or stimulation of enzyme activity (Hanke *et al.*, 1983; Assem, 1984).

In Egypt, most pesticides contamination are likely to occur in conjunction with the use of surface waters in aquaculture. It is also not unusual for aquaculturists to produce fish in ponds adjacent to fields planted in rice, cotton or a variety of other crops that require application of pesticides. Cotton fields for example, may be sprayed more than 10 times during a single growing season. If the wind is blowing strongly, or even gently from the wrong direction, the effect on an aquaculture crop can be devastating.

The present investigation aims to evaluate physiological changes in fresh water fish under the influence of different pollutants for toxicity tests in environmental water system. The biochemical and physiological indices of stress may prove to be of special value in signaling the development of sublethal abnormalities which could cause an animal population to be less efficient or effective in coping with the normal stress and strain of survival. In the present study the catfish *Clarias gariepinus* was exposed to a sublethal levels of the herbicide atrazine or the insecticide DDT for a period of several hours up to 7 days and the effect on branchial ATPases, muscle cell volume and ionic concentrations (Na^+, K^+) in plasma and muscle cells were assessed. Although the pesticide would accumulate in the tissues during exposure, the concentrations to which the fish were exposed would be environmentally realistic. The studied responses must be considered as nonspecific, as specific actions of atrazine or DDT cannot be determined; however, the range of the response should give an indication of the severity of the intoxication.

Materials and Methods

Fish

Clarias gariepinus (150-200 g) were brought to the laboratory from commercial fish pond and placed in rectangular glass tanks of 400-liters in capacity. The holding tanks received a continuous supply of non-chlorinated tap water (Temp. 25 ± 1°C).

Fish were held under these conditions for at least three weeks prior to experimentations. A photoperiod of 16 hr of light and 8 hr of dark was maintained, oxygen was never below 8 mg/liter, acidity was monitored regularly (pH 7.3 ± 0.4) using pH meter. Fish were fed on a cat fish diet (35% protein) at a rate of 3% body weight once every other day, feeding was interrupted 24 hr before the start of experiments and during their duration. Two days prior to the application of chemicals fish were transferred to a 20 liter glass test aquaria. The fish loading factor was 8 per aquarium.

Chemicals

Atrazine and DDT were obtained as technical grade from Riedel Ltd, and Stock solutions were prepared in ethanol. Toxicants were added to the glass aquaria in very small volumes of solvent. Controls contained equivalent volumes of solvent.

Experimental Design

The experiments were started by adding the ethanolic solution of atrazine or DDT to the water in test aquaria to have final sublethal concentrations of 1) 100 μg/liter atrazine, 2) 500 μg/liter atrazine, 3) 500 μg/liter DDT. The fish were sacrificed in groups of 8 after 3, 6, 9, 24, 48, 72 and 168 hr in each atrazine concentrations, and after 3, 6, 9 and 24 hr in the DDT test concentration. In a separate preexperiments, the sublethal doses were determined after the addition of the pollutants to the water in known quantities until a lethal dose was obtained (Atrazine 8000 μg/l, DDT 4000 μg/l). During exposure, water was changed completely every day.

Blood and Tissues Sampling

The fish were caught and rapidly anaesthetized in MS222 solution (50 ppm) in a separate aquarium. Immobilization was achieved within 20 sec. Blood was collected from a severed portion posterior to the head on the dorsal side. The plasma was obtained by centrifugation of the blood containing ammomium heparinized vials for 3 min at 3000 rpm and stored in a deep freezer. Individual gill arches were separated and gill filaments removed and frozen. After blood and gill sampling, the skin was removed and a piece of white expaxial muscle was taken from a definite area below the dorsal fin. It was weighed and dried to constant weight at 85°C for 48 hr and then reweighed.

Analytical Techniques

Plasma sodium was measured by flamephotometry using 5 μl plasma. Plasma potassium was determined by atomic absorption spectrophotometry using an air acetylene flame. Spectral interference between Na^+ and K^+ was minimized by adding 5% CsCl to both samples and standards. The inorganic solutes of muscle tissue were extracted from wet muscle tissue with 0.1N HNO_3 for 25 hr at 60°C. This procedure proved to be the most efficient of the several employed techniques (Assem and Hanke, 1979). No increase in ion values were found after extraction for a longer time. Na and K concentrations were again measured by flamephotometry with 5% CsCl added to avoid interference variation.

Determination of Extracellular and Intracellular Fluid Volumes

Carboxyl (^{14}C) inulin (the Radiochemical Center Amersham) was used. Each fish received 25 ul of saline solution containing 0.5 uCi tracer 3 hr before killing. The injection was given into the caudal circulation by inserting the needle into the caudal vein in the mid-line of the caudal peduncle region on the ventral surface of the fish at an angle of approximately 45°C to the vertebral column (Assem and Hanke, 1979).

Tissues of the same type as used for the chemical and water analysis were excised and vigorously rinsed for 5 sec in labelfree water before weighing. Tissue samples were prepared for liquid scintillation counting by solubilizing in 1.0 ml of 88% formic acid for two days at room temperature. 10 ml of scintillation coctail (PPO, 7 g; naphthaline, 100 g in one liter dioxan) were added to the solubilized samples. All counts measured were quench corrected. Correction for extracellular contribution of both water and ions were made on all samples taken for analysis. The actual intracellular concentration of the determined substances can be calculated for samples whose extracellular space is known using the formula: (Assem and Hanke, 1979).

$$C_i = \frac{C_t - (C_p \cdot ECS/100)}{1 - ECS/100}$$

where C_i equals intracellular concentration in mM/kg cell water, C_t equals the total tissue concentration of the substances in mM/kg tissue water, Cp equals the plasma concentration of the substances in mM/L, and ECS equals the extracellular inulin space in percent (%).

The efficiency of inulin (^{14}C) as a marker for extracellular space was determined by using polyethylene glycol (^{14}C-PEG), as an extracellular space marker (Schmidt-Nielsen *et al.*, 1972). No significant difference was observed between extracellular space measured using ^{14}C-inulin or ^{14}C-PEG.

Enzyme Assays

Na^+ - K^+ - Mg^{2+} ATPase

The gill filament of the first gill arch, was homogenized with 12 complete strokes at 350-400 rpm in ice. The homogenizing medium composed of 70 mM Na_2 EDTA, 300 mM sucrose and 10mM 2-mercaptoethanol in 100 mM imidazol-HCl buffer at pH 7.2. The $Na^+/K^+/Mg^{++}$-ATPase activity in 40-80 μg homogenate protein was determined in 0.5 ml reagent mixture containing 240 mM NaCl, 120 mM KCl, 20 mM $MgCl_2$ and 10 mM Na_2 ATP in 100 mM imidazol-HCl buffer at 37°C and pH 7.2, for 30 min. Residual or Mg^{++}-ATPase activity was determined in a similar system from which KCl was omitted, in the presence of 0.5 mM ouabain. The reaction was terminated by the addition of 1.0 ml ice cold 10% trichloroacetic acid. After centrifugation at 800 g = 25000 rpm for 10 min, the inorganic phosphate liberated from the substrate in the supernatant was determined by the method of Fiske and Subbarow (1925). The specific activity of the enzyme was calculated as the difference in rate of phosphate release between the two reagent mixtures, per mg protein. Protein levels

were determined by the method of Lowry *et al.* (1951) using bovine serum albumin as standard.

Statistical Analysis

Two-way analysis of variance was done on the data obtained. Where significant differences were evident, differences between the means were assessed statistically using student's t-test. The results were demonstrated as percentage of changes vs controls (100%), and a triangle was added when the t-test of the original values (not the relative values) indicated that the means were statistically significant ($P < 0.05$).

Results

Effects on the Na^+-K^+ Dependent ATPase

The results of in vivo effects of atrazine on the gill enzyme specific activity are demonstrated in Fig. (1). While an inhibition of the enzyme activity was observed at the lower concentration (100 μg/liter), an activation was measured at the higher concentration. This reaction of the enzyme to the presence of atrazine was followed by a significant and positively increasing activation of the enzyme at both concentrations from exposure hr 24 and which lasted throughout the rest of the experimental time. Recovery to normal control levels after 168 hr was not recorded.

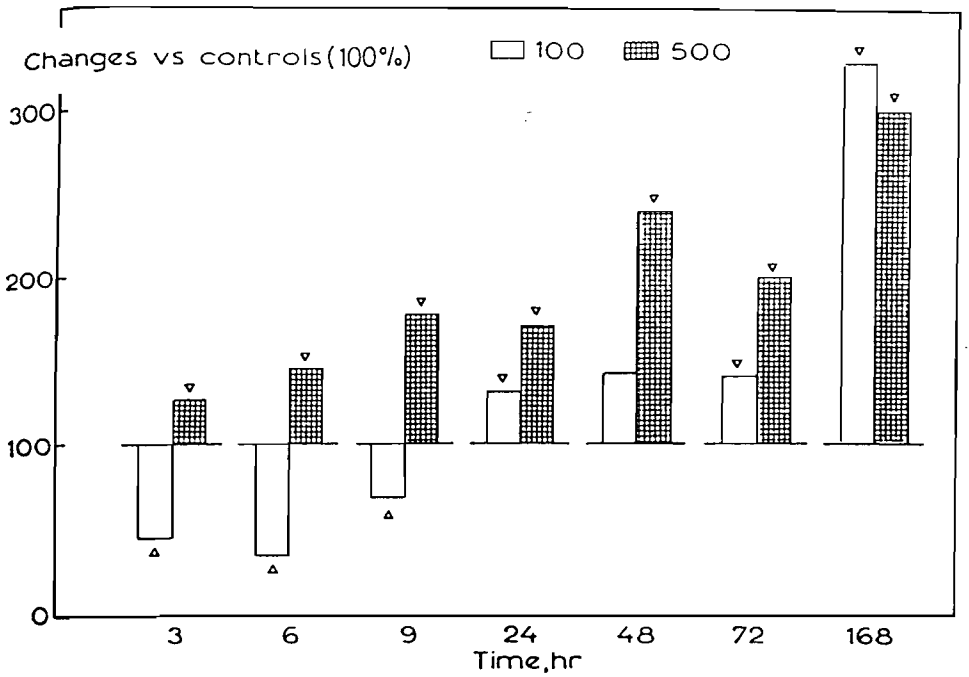

FIG. 1. Changes of gill Na^+-K^+ ATPase activity level in percentage of controls during 168 hr of exposure to 100, 500 μg/l atrazine.

The data in Fig. (2) shows the changes in Na^+. K^+-ATPase specific activity during short exposure to 500 μg/liter DDT. A rapid strong and highly significant ($P < 0.01$) increase during the first 3 hr was followed by recovery to control level during the rest of the experimental time.

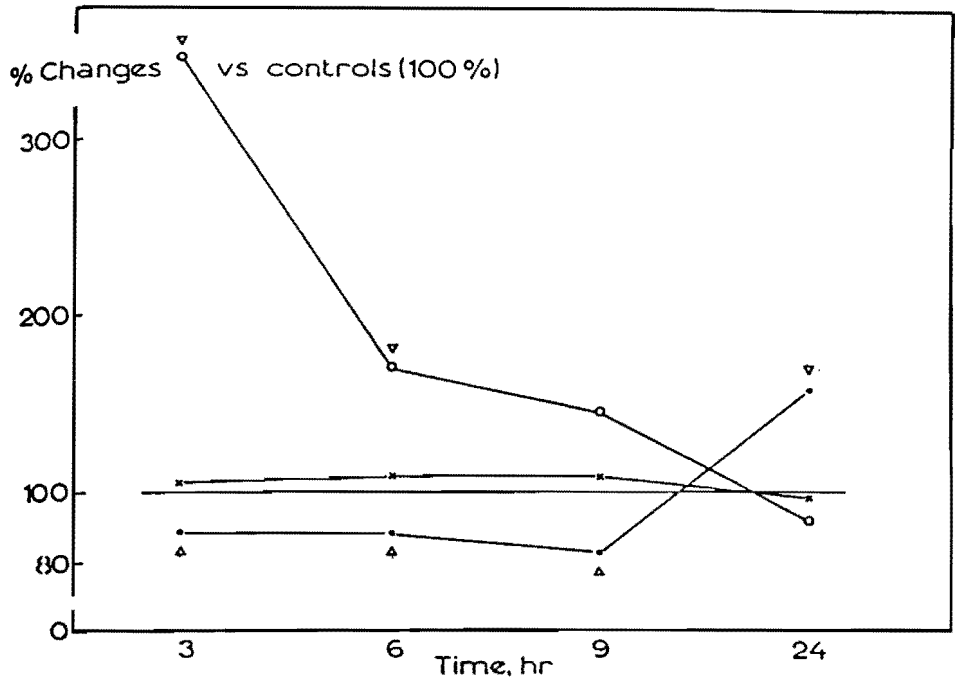

FIG. 2. Correlation of gill Na^+-K^+ ATPase ○——○, Mg^+ ATPase ●——● and protein concentration ×——× in percentage of controls during 24 hr of exposure to 500 μg/liter DDT.

Effects on the Ouabain Insensitive Mg^{++} Stimulated ATPase

The alteration in Mg^{++}-ATPase specific activity during exposure to atrazine are presented in Fig. (3). After a short initial but highly significant ($P < 0.025$) decrease at the lower concentration an increase was recorded from exposure hr 6 onwards. The enzyme was activated in fish exposed to the higher concentration (500 μg/liter) after hr 48.

In experiments with DDT, the activity of the enzyme was significantly inhibited in fish during the first 9 hr ($P < 0.005$). The subsequent activation of the enzyme by exposure hr 24 was also highly significant ($P < 0.001$) (Fig. 2).

Effects on Branchial Protein Concentrations

While gill protein concentration was increased during exposure to the lower atrazine concentration (100 μg/liter), with remarkable decrease at 24 and 48 hr, it decreased in fish exposed to its higher dose (500 μg/liter) throughout the whole period

of exposure. Recovery to normal controls level after 168 hr was not recorded at both atrazine concentrations (Fig. 4).

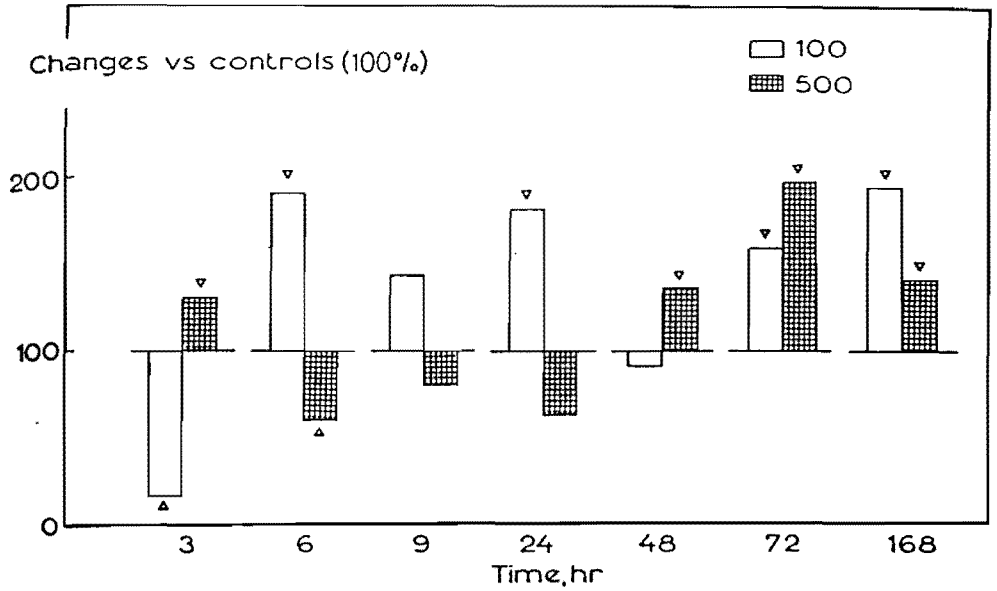

FIG. 3. Changes of gill Mg^{++} ATPase level in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

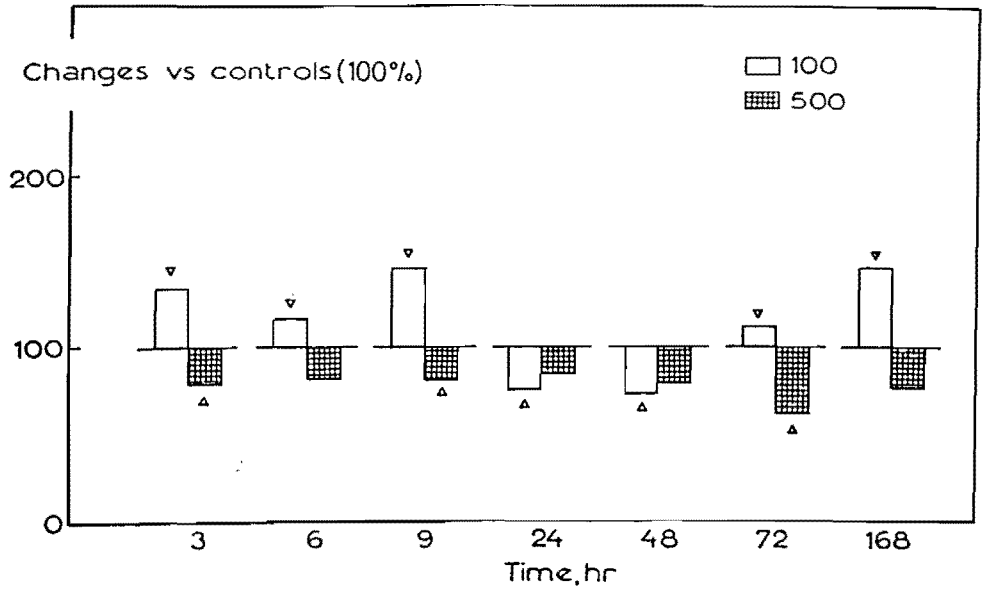

FIG. 4. Changes of gill protein content in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

In DDT experiments changes in the gill protein concentration during exposure to 500 μg/liter DDT were not statistically significant from control values (Fig. 2).

Plasma Na^+ and K^+ Concentrations

Exposure to the lower concentration of atrazine resulted in a short decrease of plasma Na^+ at 3 hr. Thereafter, it tended to increase by exposure hr 6 and 72. The changes in Na^+ concentration during the rest of experimental time were not statistically significant (although the decrease by exposure hr 24 was strong, it still not significant). The significant changes of Na^+ levels in plasma during exposure to the higher atrazine concentration were ranged from an increase by exposure hr 3, 6 and 72, to a decrease by exposure hr 24 and 168 (Fig. 5).

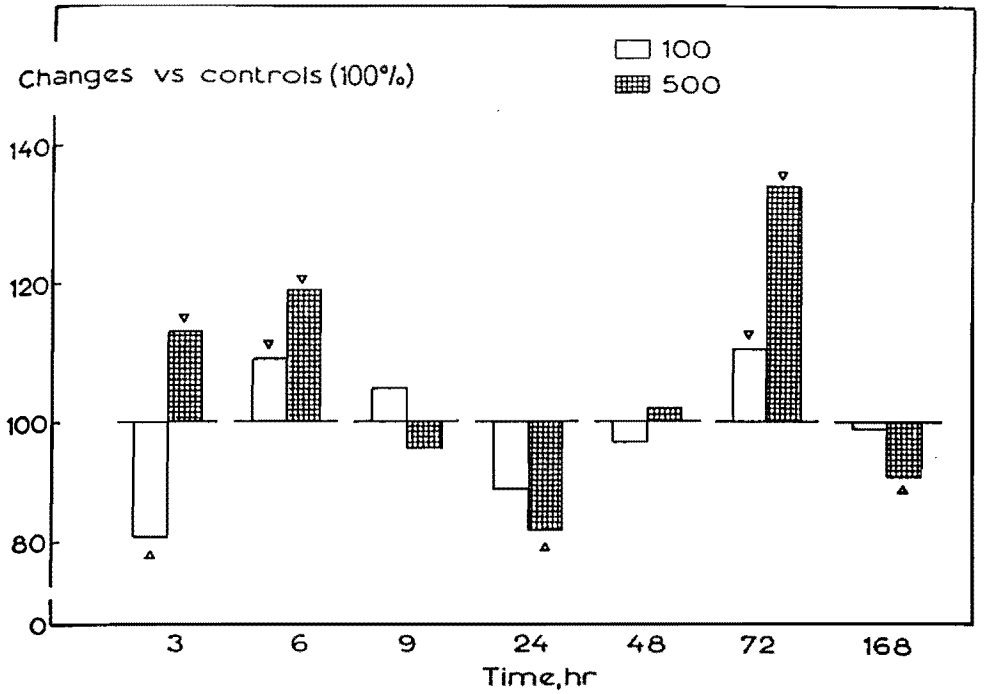

FIG. 5. Changes of plasma Na^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

The changes in plasma potassium concentrations during exposure to atrazine were not dose-dependent (Fig. 6). These changes showed the same pattern at both concentrations and ranged from a decrease by exposure hr 3, 6, 24 and 168 to an increase by exposure hr 48 and 72. All these changes were significant. In experiment with DDT exposure to 500 μg/liter produced an initial decrease in plasma sodium level by test period 3, followed by a short increase (6 hr) then recovery to normal controls level. The reduction in plasma potassium concentrations observed throughout the whole experimental time were significant (Fig. 7).

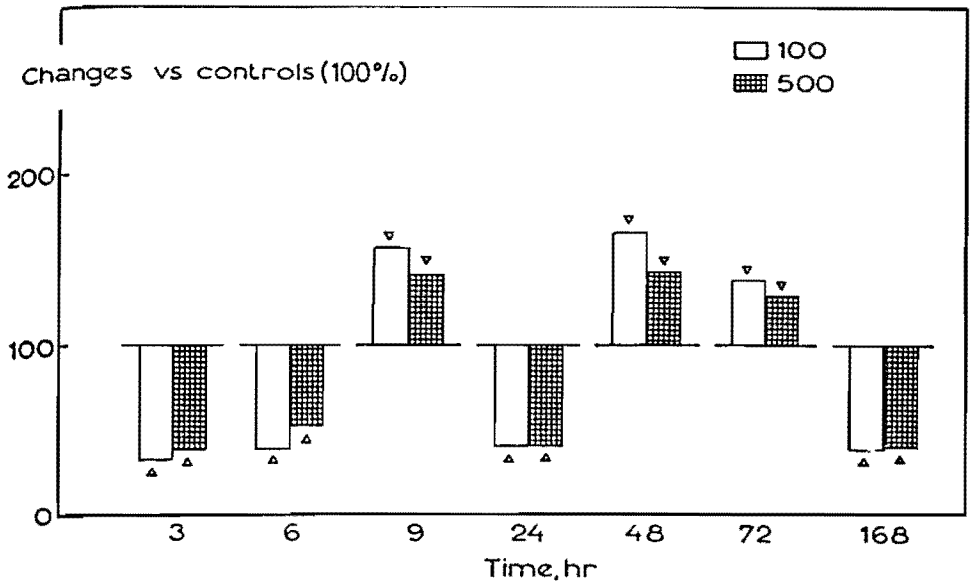

FIG. 6. Changes of plasma K^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

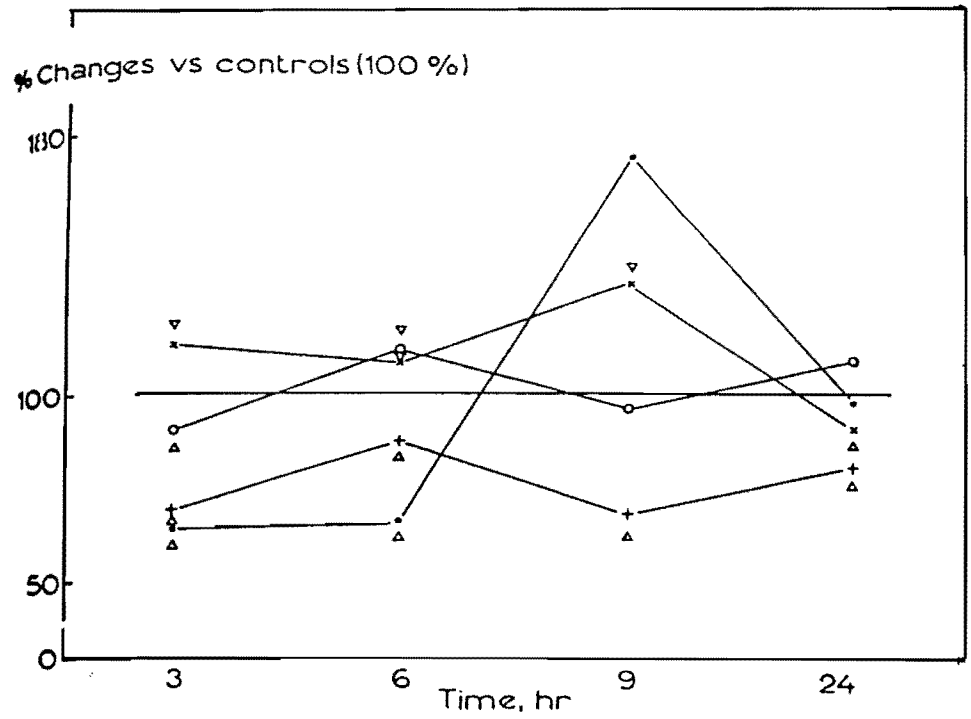

FIG. 7. Correlation of plasma Na^+ concentration ○——○, K^+ concentration +——+, muscle (ICS) Na^+ content ●——● and muscle (ICS) K^+ content ×——× in percentage of controls during 24 hr of exposure to 500 μg/liter DDT.

Muscle Intracellular Na^+ and K^+ Concentrations

The changes in cellular Na^+ concentration during exposure to atrazine are demonstrated in Fig. (8). At the lower concentration of atrazine (100 μg/liter) a significant reduction in Na^+ was measured from the beginning of the experiment till exposure hr 9. Thereafter it tends to increase significantly higher than the control with a maximum at 48 hr. Except for the condition at 3 hr, a more or less similar changes were observed in fish exposed to atrazine at the higher concentration (500 μg/liter).

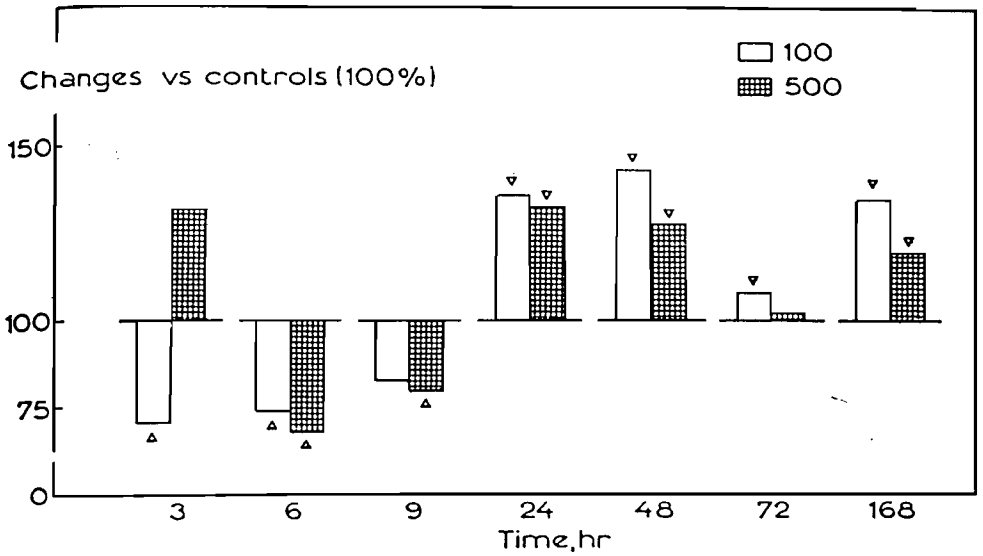

FIG. 8. Changes of intracellular Na^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

The significant changes in cellular potassium during exposure to the lower atrazine concentration were ranged from an increase by exposure hr 3, 9 and 168 to a decrease by exposure hr 6, 48 and 72, the magnitudes of these changes were similar. At the higher atrazine concentration the cellular potassium concentration decreased significantly by exposure hr 3 and 6 then mainly increased to a constant level from exposure hr 9 onwards (Fig. 9). Exposure to DDT resulted in a rapid decrease in cellular sodium concentration by exposure hr 3 and 6. After 9 hr exposure its concentration increased significantly but recovered to nearly normal level at 24 hr exposure. In contrast cellular potassium concentration was increased during the first 9 hr in DDT, then decreased to a level which was significantly ($P < 0.001$) lower than the controls (Fig. 7).

Muscle Intracellular Space (ICS)

Atrazine at both concentrations produced a significant rapid and dose-dependent, cell swelling, being higher at the higher concentration. This initial change was fol-

lowed by cellular shrinkage from the 9th hr onward. Till the end of exposure time (168 hr), no indication of cell volume recovery was recorded (Fig. 10). In vivo exposure to DDT produced a highly significant ($P < 0.005$) cellular dehydration which increased with time (Fig. 11).

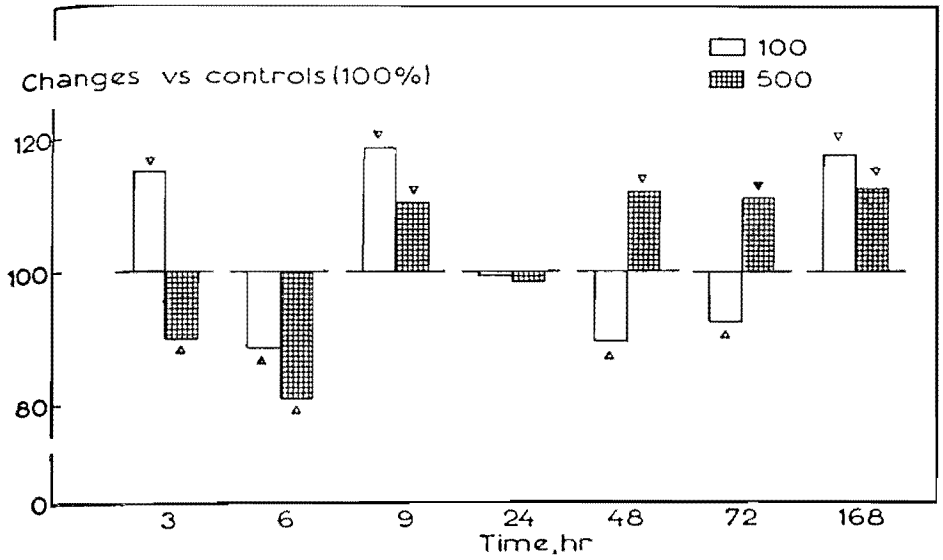

FIG. 9. Changes of intracellular K^+ concentration in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

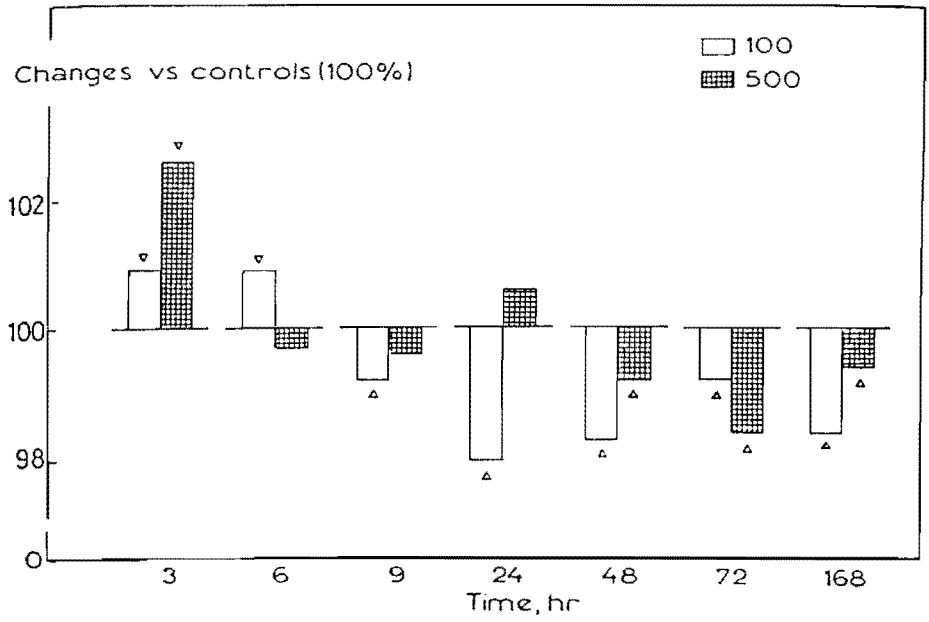

FIG. 10. Changes of muscle cell volume in percentage of controls during 168 hr of exposure to 100, 500 μg/liter atrazine.

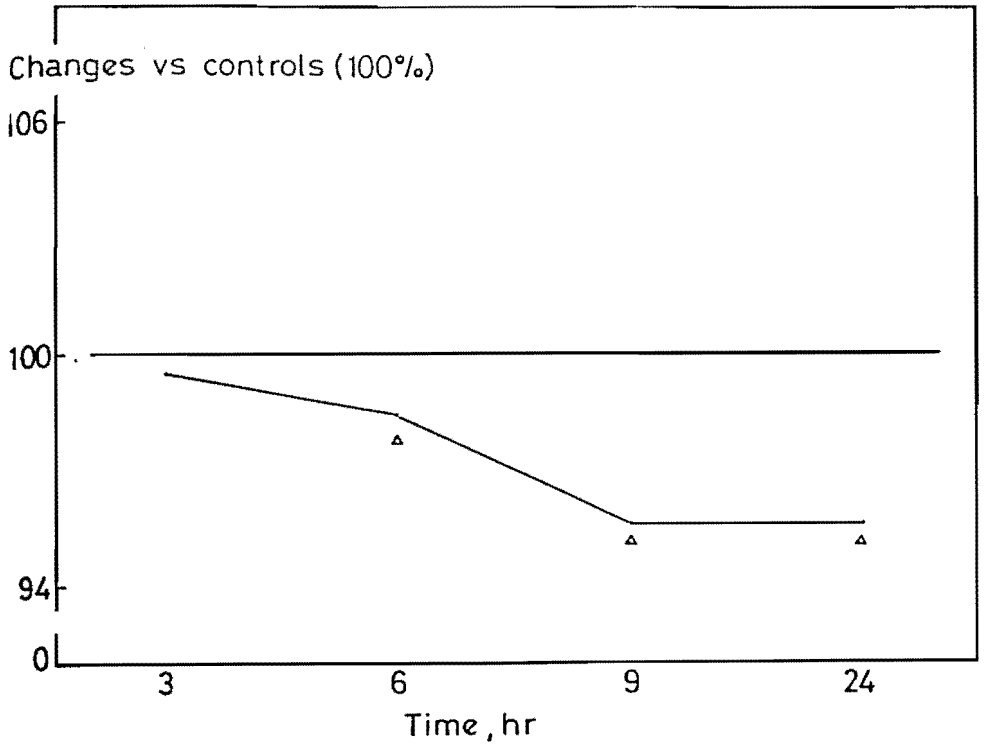

FIG. 11. Changes of muscle cell volume in percentage of controls during 24 hr of exposure to 500 μg/liter DDT.

Discussion

In the present study the Na^+, K^+-ATPase was sensitive to atrazine at both concentrations. At the higher concentration, the recorded increase of enzyme activity was accompanied by an increase in plasma sodium level, which may be due to an enhanced branchial sodium transport. The simultaneous reduction of the level of plasma potassium would indicate osmoregulatory problems. A short activation of gill Na^+, K^+-ATPase was also recorded when the salt water adapted carp, *Cyprinus carpio*, was exposed to 1000 μg/liter atrazine (Hanke *et al.*, 1983). The present results are not in accordance with those obtained by Assem (1984) using the same dose of atrazine (500 μg/liter) and the carp as experimental fish. It seems that the increase in enzyme activity is due to an activation of the existed enzyme units rather than de novo synthesis, the parallel decrease of gill total protein concentration may support this assumption. An opposite response was observed during exposure to the lower atrazine concentration where the enzyme was inhibited. The increase of branchial total protein concentration occurred concomitantly may indicate enzyme synthesis to compensate for inhibitory effect of atrazine.

The initial reduction of Na^+-K^+-ATPase specific activity was accompanied by a significant decrease in the level of plasma Na^+ and K^+. Likewise, Renfro *et al.* (1974) attributed the observed mercury-induced depression of Na^+ and K^+ transport in several osmoregulatory organs of different fresh and sea water teleosts to an inhibition of the Na^+, K^+-ATPase activity. On the other hand our observations seems to be at variance with the conclusions obtained by several other investigators. For example, Bouquegneau (1977) related elevated Na^+ and Cl^- levels in the plasma of sea water adapted eels, *Anguilla anguilla,* to an inhibition of the Na^+, K^+-ATPase activity of the gills. Lock *et al.* (1981) have also found a correlation between inhibition of the Na^+, K^+-ATPase in the gill of *Salmo gairdneri,* and its osmoregulation upon exposured to mercuric chloride and methylmercuric chloride but attributed this relation primarily to changes in the permeability characteristics of the gills for water. This may explain the absence of a correlation between the increase of the gill enzyme activity and plasma Na^+, K^+ levels of *Clarias gariepinus* exposed to both atrazine concentrations from the second day onwards.

From our previous discussion it appears therefore that Na^+, K^+-ATPase activation (500 μg/liter atrazine) or inhibition (100 μg/liter) may have value as an indicator of stress caused by atrazine. The disturbance of osmoregulation of the fish *Clarias garienpinus* exposed to atrazine at both concentrations affect also the level of the active cations at the muscle site.

The most important changes in the activity of gill Mg^{++} ATPase were represented by an activation of the enzyme from the 72th exposure hour onwards at both concentrations of atrazine. A similar simultaneous increase of both Na^+, K^+ and Mg^{++} ATPase was recorded by Watson and Beamish (1980) when they exposed the rainbow *Salmo gairdneri* to different zinc concentrations. They attributed their results to the zinc competition for Mg^{++} binding site in the gill epithelial membrane which might affect an increase in branchial permeability. Assuming that atrazine may act as Zn, the general augmentation of gill ATPase activities (Na^+, K^+ and Mg^{++}) could result from the diffusional loss of ions due to increased gill permeability. The ultimate reduction in ion concentration could, in turn, act as a signal for gill ATPase to increase the absorption rate of electrolytes.

The gill Na^+, K^+-ATPase of *Clarias gariepinus* was also extremely sensitive to DDT. The exposure of the fish to 500 μg/liter DDT produced a strong activation of gill Na^+, K^+-ATPase by exposure hr 3, then it decreased gradually with time to normal control level. Meanwhile, gill total protein remain constant throughout the whole experimental time which may indicate an activation of the present enzyme units. This result confirms similar reports by Khalifa (1989); and by Jowett *et al.* (1978), but are at odds with those of Hanke *et al.*, (1983); and Assem (1984) who reported striking inhibition by DDT of the Na^+, K^+-ATPase of the carp, *Cyprinus carpio.* The discrepancy may indicate that the fish, *Clarias gariepinus,* is less sensitive to DDT than the carp.

Of particular interest was the reduction of plasma Na^+ level by DDT treatment. This indicate that the fish's ability to maintain the sodium ion concentration at a con-

stant level above that of the external medium, had broken down. It is difficult to explain these results because we expected an increase of plasma Na^+ instead of the measured decrease, the reduction of intracellular Na^+ further complicated this matter. A similar paradox was also observed by many investigators who failed to find a relation between the changes of gill Na^+, K^+-ATPase activity and the ionic balance of the studied fish (Neufield and Pritchard, 1979a, b; Watson and Beamish, 1980; Boese *et al.*, 1982; Khalifa, 1989).

The simultaneous opposite changes of potassium concentrations in plasma and muscle cells indicate an uptake or release of the ion between intracellular and extracellular spaces. It may also indicate that potassium ion balance remained unaffected by DDT treatment.

Activity of Mg^{++}-ATPase was decreased after the fish were exposed to 500 µg/liter DDT. Inhibition of gill Mg^{++}-ATPase by exposure to DDT was observed by many investigators (Hanke *et al.*, 1983; Leadman *et al.*, 1974; Miller and Kinter, 1977; Jowett *et al.*, 1981). Assem (1984) has found no significant effects of DDT on Mg^{++}-ATPase activity in the gill of the carp, *Cyprinus carpio*.

In conclusion, investigations to date indicate that the effects of the environmental pollutants upon gill ATPase activities (Na^+, K^+ and Mg^+) and osmotic and ionic balance are varied among organisms. Many factors may influence this interaction including the chemical composition of the cell membrane, molecular characteristics of the enzyme binding of the pollutant to macromolecules and the physiological nature of the organism. Certainly, the osmoregulatory mechanism may be adversely affected by xenobiotics but other cell membrane processes may be altered as well. A review of the membrane theory of toxicity has been prepared by Kinter and Pritchard (1977). Even though the slight alteration of any one process may appear insignificant, the combination of effects among several processes may increase stress in such a way that the organism is less able to cope with various forms of adversity.

References

Abo-Hegab, S. and **Hanke, W.** (1981) Electrolyte changes and volume regulatory processes in Carp, *Cyprinus carpio*, during osmotic stress. *Comp. Biochem. Physiol.* **71A:** 157-164.

——— (1986) Electrolyte changes, cell volume regulation and hormonal influences during acclimation of rainbow trout, *Salmo gairdneri* to salt water. *Comp. Biochem. Physiol.* **83A;** 47-52.

Assem, H. (1984) Potential use of physiological changes as indices of stress in the teleost, *Cyprinus carpio*. *Bull. Inst. Oceanogr. Fish. A.R.E.* **10:** 111-121.

——— and **Hanke, W.** (1979) Volume regulation of muscle cells in the euryhaline teleost, *Tilapia mossambica*. *Comp. Biochem. Physiol.* **64A:** 17-23.

Boese, B.L., Johnson, V.C., Champan, D.E. and **Ridlington, J.W.** (1982). Effects of petroleum refinery wastewater exposure on gill ATPase and selected blood parameters in the Pacific staghorn sculpin *Leptocottus armatus*. *Comp. Biochem. Physiol.* **71C:** 63-67.

Bouquegneau, J.M. (1977) ATPase activity in mercury intoxicated eels. *Experientia* **33:** 941-943.

Fiske, C. and **Subbarow, Y.** (1925) The colorimetric determination of phosphorus. *J. Biol. Chem.* **66:** 375-400.

Gluth, G. and **Hanke, W.** (1983) The effect of temperature on physiological changes in carp, *Cyprinus carpio* L., induced by phenol. *Ecotoxicol. Environm. Safety* **7,** 373-389.

Hanke, W., Gulth, G., Bubel, H. and **Muller, R.** (1983) Physiological changes in carps induced by pollution. *Ecotox. Environ. Safety* **7:** 229-241.

Jampol, L.M. and **Epstein, F.H.** (1970) Sodium-potassium activated adenosine triphosphatase and osmotic regulation by fishes. *Am. J. Physiol.* **218:** 607-611.

Jowett, P.E., Rhead, M.M. and **Bayne, B.L.** (1978) In vitro changes in the activity of ATPases in the gills of *Carcinus maenas* exposed to various concentrations of p,p'-DDT. *Envir. Pollut.* **17:** 1-6.

——— (1981) In vivo changes in the activity of gill ATPase and haemolymph ions of *Carcinus maenas* exposed to p,p'-DDT and reduced salinities. *Comp. Biochem. Physiol.* **69C:** 399-402.

Khalifa, A. (1989) *Physiological responses of the fish Clarias lazera to some organic chemicals.* M.Sc. Thesis, Fac. Sc., Alexandria University, 185 p.

Kinter, W.B. and **Pritchard, J.B.** (1977) Altered permeability of cell membranes. In: *Handbook of Environmental Physiology - 1. Physical and Chemical Agents* (Edited by **Lee, D.H.K., Falk, H.L. Murphy, S.D.** and **Geiger, S.R.**), American Physiological Society, Washington, D.C., pp. 563-576.

Leadman, T.P., Cambell, R.D. and **Johnson, D.W.** (1974). Osmoregulatory responses to DDT and varying salinities in *Salmo gairdneri*. I. Gill Na^+ ATPase. *Comp. Biochem. Physiol.* **49A:** 197-205.

Lock, R.A.C., Cruijsen, P.M.J.M. and **Van Overbeeke, A.P.** (1981) Effects of mercuric chloride on the osmoregulatory function of the gills in rainbow trout, *Salmo gairdneri*, Richardson. *Comp. Biochem. Physiol.* **68C:** 151-159.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and **Randall, R.J.** (1951) Protein measurement with the folin phenol reagent. *Biol. Chem.* **193:** 265-275.

Miller, D.S. and **Kinter, W.B.** (1977) DDT inhibits nutrients absorption and osmoregulatory function in *Fundulus heteroclitus*. In: *Physiological Responses of Marine Biota to Pollutants* (Edited by **Vernberg, F.J., Calabrese, A., Thurberg, F.P.** and **Vernberg, W.B.**), Academic Press, New York, pp. 63-74.

Neufield, G.J. and **Pritchard, J.B.** (1979a) Osmoregulation and gill Na^+-K^+-ATPase in the rock crab, *Cancer irroratus*, response to DDT. *Comp. Biochem. Physiol.* **62C:** 165-172.

——— (1979b) An assessment of DDT toxicity on osmoregulation and gill Na, K-ATPase activity in the blue crab. In: *Proceeding of the Symposium on Aquatic Toxicology* (Edited by **Marking, L.L.** and **Kimerle, R.A.**), American Society for Testing and Materials, pp. 23-34.

Renfro, J.L., Schmidt-Nielsen, B., Miller, D., Enos, D. and **Allen, J.** (1974) Methylmercury and inorganic mercury; uptake, distribution and effect of osmoregulatory mechanisms in fishes. In: *Pollution and Physiology of Marine Organisms* (Edited by **Vernberg, F.J.** and **Vernberg, W.B.**), Academic Press, New York, pp. 101-122.

Schmidt-Nelson, B., Renfro, J.L. and **Benos, D.** (1972) Estimation of extracellular space and intracellular ion concentration in osmoconformers, hypo- and hyper-osmoregulators. *Bull. Mt. Desert. Isl. Biol. Lab.* **12:** 99-104.

Watson, T.A. and **Beamish, F.W.H.** (1980) Effects of zinc on branchial ATPase activity in vivo in rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **66C:** 77-82.

Watson, T.A. and **Beamish, F.W.H.** (1981) The effect of zinc on branchial adenosine triphosphatase enzymes in vitro from rainbow trout, *Salmo gairdneri*. *Comp. Biochem. Physiol.* **68C:** 167-173.

Zaugg, W.S. and **Mclain, L.R.** (1970) Adenosinetriphosphatase activity in gills of Salmonids: Seasonal variations and salt water influence in coho salmon (*Oncorhynchus kisutch*). *Comp. Biochem. Physiol.* **35:** 587-596.

التغيرات الحيوية في نشاط إنزيم الأدينوسين تراي فوسفاتاز وحجم خلايا العضلات وتركيز الأيونات في بلازما الدم وخلايا العضلات عند تعريض أسماك القط الأفريقي لتركيزات مختلفة تحت قاتلة من مبيد الحشائش الأترازين أو مبيد الحشرات د. د. ت.

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المستخلص . تم تعريض أسماك القط الأفريقي لتركيزات مختلفة تحت قاتلة من مبيد الحشائش الأترازين أو مبيد الحشرات د.د.ت. ، وذلك بهدف تقييم استخدام بعض التغيرات الفسيولوجية التي تحدث في الأسماك كدليل على تلوث المياه . والجدير بالذكر أن مبيد الحشرات د.د.ت. رغم تحريم استخدامه دوليًا ، مازال مستخدمًا في بعض الدول نظرًا لانخفاض سعره وأثره الفعال مقارنة بالمبيدات الفوسفورية الأقل ضررًا والأكثر تكلفة .

ولقد لوحظ أن التغيرات التي حدثت في نشاط الأنزيم في خلايا خياشيم تلك الأسماك يمكن الاعتماد عليها كدليل على وجود مادة سامة في البيئة ، كما لوحظ تغير له مغذي إحصائي كبير في مكونات جسم الأسماك من عنصري الصوديوم والبوتاسيوم ، وكذلك تركيز هذين العنصرين في بلازما الدم .

ويمكن التوصية بالاعتماد على التغيرات الفسيولوجية التي حدثت في الأعضاء التي تمت دراستها كدليل على وجود مواد غريبة في بيئة الأسماك ، على أن يكون المعمل مجهز بالإمكانيات اللازمة لإجراء التحليل ، وخاصة نشاط الأنزيم .