J. Union Arab Biol, Cairo
http://www mideastnet. Com arabbiologists
Email: union biologists@arabmarketcom
Vol. (12A) Zoology 127-143, Nov. 99
Rec. 30/9/99

ISSN 1110-5372
6th International Conference.
8-10 Nov. 99

MODULATION OF THE HUMORAL IMMUNE RESPONSE OF RAINBOW TROUT EXPOSED TO ECOTOXIC STRESS FACTORS

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Key words: **Ecotoxic stress-humoral immune response-Rainbow trout**

ABSTRACT

Unacceptable risks are posed to ecological receptors from chemicals as well as many other pollutants. Chemical contamination is considered a major ecological stress factor on fresh water fish. Stress can cause a reduction in food intake, impair growth, reproduction and immunity. Copper sulfate, Malathion and Paraquat, as industrial overuse and agricultural pesticides, can cause extensive contamination to aquatic environments. This study is concerned with the determination of the modulating effect of acute sublethal doses of the three chemicals in the fresh water fish rainbow trout (*Onchorynchus mykiss*). A monoclonal antibody dependent indirect enzyme linked immunosorbant assay (ELISA) was used. The evaluation of the immune modulation was based on the determination of the IgM titers following KLH immunization. Significant changes in the IgM response patterns indicated suppression and/or enhancement of the immune system of fish. Such data could be of value for preliminary problem formulation and ecological effects evaluation in ecosystems.

INTRODUCTION

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ABSTRACT

Unacceptable risks are posed to ecological receptors from chemicals as well as many other pollutants. Chemical contamination is considered a major ecological stress factor on fresh water fish. Stress can cause a reduction in feed intake, impair growth, reproduction and immunity. Copper sulfate, Malathion and Paraquat, as industrial overuse and agricultural pesticides, can cause extensive contamination to aquatic environments. This study is concerned with the determination of the modulating effect of acute sublethal doses of the three chemicals in the fresh water fish rainbow trout (*Onchorynchus mykiss*). A monoclonal antibody dependent indirect enzyme linked immunosorbant assay (ELISE) was used. The evaluation of the immune modulation was based on the determination the IgM titers in correlation to KLH immunization. Significant changes in the IgM patterns indicated suppression and/or enhancement of the immune system of fish. Such data could be of value for preliminary problem formulation and ecological effects evaluation in ecosystems.

INTRODUCTION

The use of fresh-water bodies for domestic and commercial activities is beneficial for all residents. Unfortunately, these water bodies are susceptible to chemical contamination (U S Department of Agriculture, 1968 and Eisler, R., 1991) from industrial and agricultural activities such as accidental spills, drainage from washing and cleaning of spray equipment and pesticide

containers, drift from spraying operations or runoff from newly treated fields. Chemical contamination is considered a major ecological stress factor on non-target organisms including fresh water fish.

The role of the ecological risk assessment is to: determine whether unacceptable risks are posed to ecological receptors from chemical stressors, derive contaminant levels which would not pose unacceptable risks and provide the information necessary to make a risk management decision concerning the practical need and extent of remedial action (Laws, 1994). The ecological risk assessment is in a beginning phase of development and therefore exists in a very dynamic state. Guidance is limited and there is uncertainty concerning the roles and processes of ecological risk assessment in the different programs.

By diffinition, stress is considered a change in homeostasis that can cause a reduction in feed intake, impair growth, reproduction and immunity (Van Muiswinkle et al., 1985). Pesticides and other chemical overuse, as stress factors, may not cause an apparent disturbance in aquatic and terrestrial environments, but adverse affects have been observed in non-target organisms including fresh-water fish (Kimbrough and Gaines, 1970; Dueck *et al*., 1987 and Nor, 1987). Copper sulfate [$CuSO_4$], Malathion [ML, (O, O-dimethyl-S-1, 2-di-ethoxycarbamyl ethylphos-phor–dithioate)] and Paraquat [PQ, (1, 1'-dimethyl-4, 4'-bipyridinium dichloride)] can cause extensive contamination to aquatic environments and accordingly, may cause stress effects ranging from changes at the molecular level to population and community levels (Segner and Braunbec, 1988 and Fairbairn *et al*., 1995).

Sublethal doses of ecotoxic stress factors on fish can have detrimental effects upon immune system structures and/or functions that may ultimately can be as harmful as direct toxic doses (Spitsbergen *et al.*, 1986 and Thuvander, 1990) . The aim of the present study is to determine the modulating effect of acute sublethal doses of $CuSO_4$, ML and PQ on the humoral immune responce of the fresh water fish rainbow trout. The evaluation of such modulation, with the rarity of immunological information related to these stress factors, could be of value for preliminary problem formulation and ecological effects evaluation.

MATERIAL AND METHODS

Fish:

Immature rainbow trout (*Onchorynchus mykiss*) with an average weight of 90 (60–120) g and average length of 20 (18–22) cm were obtained from an Utah local hatchery (Utah Division of Wildlife Resources, Springville Hatchery, Utah, USA). Fish were kept in glass aquaria supplied with aerated, charcoal-filtered circulating tap water (0.4 L/min) at a temperature of 15°C, under a 12-hr light / 12-hr dark photoperiod. The fish were fed on homogenous commercial trout pellet (Ewos), and acclimatized to laboratory conditions for a minimum of one week before the experiments were performed.

Experimental design:

The acute toxicity of a chemical to fish is usually expressed as 96 hr LC_{50} in ppm. Selection of the used $CuSO_4$, ML and PQ sublethal concentrations was led by the available reports of acute toxicity rating classification and the previously determined levels of LC_{50} on fresh water fish (Mayer and Ellersieck, 1986). $CuSO_4$ was classified as slightly toxic of 10-100 ppm 96 hr LC_{50} but with high acute screening value. ML and PQ were classified as highly toxic of 0.1-1.0 ppm 96 hr LC_{50} but with low acute screening value. Befor the main experiment was performed, the chosen sublethal doses of $CuSO_4$, PQ and ML and their two folds were tested to determine their lethality. Each of the concentrations was tested on five fish for a period of one week. None of the fish was found dead. Fish were exposed to each of low and high concentrations of $CuSO_4$ (low = 0.01 and high = 1.0 ppm), ML (low = 0.0001 and high = 0.01 ppm) and PQ (low = 0.0001 and high = 0.01 ppm) by immersion in the aquaria for 96 hours. Additionally two control groups (each of five fish) were included: nonimmunized / nontreated control fish (negative control) and immunized / nontreated control fish (positive control).

Keyhole limpet hemocyanin (KLH, Sigma, USA) immunization was performed via intraperitoneal (ip) injection of 100 μg/g wet fish. The booster dose of KLH was given, at the same dose and route, after 3 weeks following the first immunization.

Blood sampling:

Blood samples were collected from five fish of each fish group at time of first immunization and at one week interval for 7 weeks. In addition correlated blood samples were collected from fish of the negative control group. At time of sampling, fish were anaesthetized with MS-222 and blood was collected from the ventral aorta into none heparinized vacutainer tubes. The tubes were kept at 4°C overnight then centrifuged at 300 g for 10 min to separat serum. Collected samples were initially diluted 1:100 in PBS containing 2% none fat dry milk and 0.5 % Tween-20 (dilution buffer). Starting with this dilution, twelve serial 2-fold dilutions (1:0.2 $x10^3$ - 1:409.6 $x10^3$ were prepared in the same buffer. All dilutions were prepared immediatly before the detection of their IgM titers.

Enzyme linked immunosorbent assay (ELISA):

Levels of humoral antibodies to KLH were measured by indirect ELISA technique. One hundred μl of 100 μg KLH/ml is added to each of the wells of polystyrene microtitre plates (Nunc II Immunoplate, Nunc, Denmark). Plates were incubated for one hour at 37°C. After tapiing off, free sites of the wells were immediately blocked for one hr at room temperature (rt) with 200 μl of 2% none fat dry milk in phosphate buffered saline (PBS). After blocking, plates were then washed five times with PBS containing 0.05% Tween-20 (washing buffer). Subsequently, 100 μl of each of the previously diluted fish plasma were added to each of three wells. Plates were incubated for one hr at room temperature.

After washing, 100 μl of mouse monoclonal anti- rainbow trout IgM antibody, diluted 1:100 in dilution buffer, were added to each well. After incubation period of one hour at rt, plates were rewashed as above. An aliquote of 100 μl of rabbit anti-mouse IgG antibody conjugated to horseradish peroxidase, diluted 1:5000 in dilution buffer was then added to each well. The plates were further incubated for one hour at rt and then extensively washed (ten times) with washing buffer. For coloure development, 200 μl 3,3,5,5-tetramethylbenzidine (TMB) were added per well. The reaction was stopped after 45 min with 50 μl/well of 2 M H_2SO_4. The optical density was read spectrophotometrically at 450 nm using multichannel photometer (Flow Laboratories). The highest dilutions expressing end point reaction for each group were used to express the titer of the IgM antibody levels.

Statistical evaluations:

The data were analysed for statistically significant differences between negative control and treated fish by the students's t-test. Analysis was based on comparing absorbance values, obtained at end-point titers, in relation to time laps.

RESULTS

From data depected repeatedly in tables 1, 2 and 3 the titers of negative and positive control fish expressed the following behaviour (Fig 1): negative control fish expressed IgM titers ranging from 3.2 $x10^3$ to 0.8 $x10^3$. The pattern of the titers in correlation with time laps was decreasing, as indicated by the trend line. Positive control fish expressed titers of 3.2 $x10^3$ for three weeks indicating a steady state phase followed by an increase to reach a maximum of 12.8 $x10^3$ at the third week. Afterthen, the titer declined to reach a minimum of 1.6 $x10^3$ at the fifth week. A reincrease of the titer was expressed in the sixth week reaching a value of 6.4 $x10^3$, this persists till the end of experiment. The pattern of the titers was generally increasing, as indicated by the trend line.

Fish exposed to low and high $CuSO_4$ concentrations showed changes in the IgM titers (table 1), as copmared to both negative and positive control fish. Exposure to low $CuSO_4$ concentration lowered the IgM titer at the second week (1.6 $x10^3$), followed by an increased titer during the subsequent three weeks of 6.4 $x10^3$. During the fifth and sixth weeks another increase was observed (25.6 $x10^3$) follwed by a sharp decline to reach a value of only 3.2 $x10^3$. The trend of titers was increasing as compared to that of positive control fish (Fig 2a). The IgM titer of fish exposed to the high $CuSO_4$ concentration expressed the same behavior as of above with only on exception that the titer was markedly increased (102.4 $x10^3$) at the end of experiment. The trend of the pattern was dramatically increasing, as compared to positive control fish (Fig 2a).

The IgM titers of fish exposed to low and high concentrations of ML showed an increasing trends (Fig 2b). As shown in table 2, the titers of fish exposed to low concentration of ML expressed two maxima at the third (12.8 $x10^3$) and sixth (102.4 $x10^3$) weeks. In case of fish exposed to high ML concentration, titers showed the same behavior with higher values of 51.2 $x10^3$ and 204.8 $x10^3$ (table 2) respectively. In addition, fish of the two experimental groups showed drops of their IgM titers at the last week.

Fish exposed to low concentration of PQ revealed gradual

increase in their IgM titer (table 3), reaching a maximum value of 25.6 $x10^3$ at the fourth week. After a week, a significant sudden drop was observed. A second maximum of 51.2 $x10^3$ was expressed in the sixth week, then a drop follows. Similar behaviour was recorded for fish group treated with the high concentration of PQ but with higher maxima of 51.2 $x10^3$ and 102.4 $x10^3$ (table 3) and generaly higher trend of increased IgM level (Fig 2c). Fish of the two experimental groups showed drops of their IgM titers to reach values of 12.8 $x10^3$ and 25.6 $x10^3$ at the last week.

In general, psitive control fish (Fig 1) had increased IgM titers at the third and sixth weeks. The second increase was expanded till the last week. The maximum titer of 12.8 $x10^3$ was obtained at the third week. Data depected in table 4 indicates an average value of 5.4 $x10^3$ and a doubling value of 2.8 as compared with data of the negative control fish. Table 4 shows also corresponding values in relation to fish exposure to each of the three tested ecotoxic stress factors. In relation to $CuSO_4$, fish exposed to high concentration gave a maximum IgM titer of 102.4 $x10^3$ at the last week with a doubling value of 18.3 of the average titer. Two maximal IgM peaks were shown by fish obtained ML treatment at the third and seventh weeks, independent of concentration (Fig 2a). The higher maximum (204.8 $x10^3$) was obtained with high concentration with adoubling value of the average titer of 25.9 (table 4). Similar behaviour was obtained with fish exposed to PQ, but the two maximal IgM peaks were shifted to the fourth and sixth weeks (Fig 2c). Evaluation parameters for PQ treated fish are depected also in table 4.

DISCUSSION

To detect a less pronounced antibody responce or to reveal minor modulations of the humoral response due to environmental stress factors, more sensitive method would be of value. The ELISA technique has been used in several studies of the humoral antibody in rainbow trout (Cossarini-Dunier, 1985; Hamilton *et al.,* 1986 and Thuvander *et al.*, 1987) as it is a sensitive and inexpensivebmethod which permits rapid screening of large number of samples. Furthermore, only a small amount of plasma is required from each individual. This is an important advantage in studies of the immune respomse in fish. Additionally, The use of anti rainbow trout IgM monoclonal antibody in the present study is important to reduce the background level of nonspecific interactions in the ELISA.

Immunization of rainbow trout with two doses of KLH, as a soluble protein, induced enhanced IgM secretion in the form of primary and secondry humoral responce. The primary responce represents similar phases to those of mammals (Klein, 1982) except the prolonged lag phase in the begining of IgM secretion. Background level of KLH nonspecific background titers, detected in nonimmunized fish, must be due to interaction of immunogens originally present in the fishary.

Fish exposed to low acute dose of $CuSO_4$ revealed early primary response which did not reach the level of positive control fish and marked with no decline phase. The secondry responce was enhanced but the sudden drop of the last value indicates a failieur of the immune system to offer prolonged humoral protection. This is in contradiction with the finding of Thuvander (1990) who stated that the greatest proportion of seropositive fish were found 7 weeks post vaccination. Hetrick *et al.* (1982) revealed that striped bass exposed to copper compounds for 96 hr increased the susceptibility of fish to bacterial diseases.

High acute dose of $CuSO_4$ caused disaperance of the lag phase at the begining of immunization, followed by enhanced primary responce marked by elevated titers of fish IgM. The secondary response was accumulative with sharp increase at the last week of expriment. This indicates hypoactivity of the down loading limb of the immune responce. Such charachteristic behavior of persistant IgM increase could lead to fish autoimmunefunctions. Copper, as a metal, blocks the active sites of antibody molecules and disturb the metabolism (O'Neill, 1981b). Also, reactivation of the humoral response is sometimes dangerous, especially when soluble antigens are employed. Antigen might fix to tissue cells, and upon boosting, refix to newly injected antigen causing anaphylactic shock (Barrett, 1983).

Fish exposed to acute doses of ML showed similar IgM primary and secondary patterns when compared with positive control fish in relation to time laps and secretion phases. The marked observation of elevated titers, wheter of primary or secondary responses, reflect overstimulation of the fish humoral activity. Fish exposed acute doses of PQ for revealed no indication of the lag phase, but a steady state was remarkably expressed for a whole week. The maxima of the primary response were delayed independent on the dose. The patterns of the secondary response were similar to those of fish treated with ML. These findings contradict with several reports (Zeeman and Brindley, 1975; Areechon and Plumb, 1990; Cossarini-Dunier *et al*., 1991). From

the very limited work that has been done on rainbow trout (Thuvander *et al.,* 1987 and Thuvander, 1989), pesticides may exhibit immunotoxicity on immune cells and correspondingly the secretion of IgM is a matter of variability. Up to date, it is somewhat surprising that so little is known about how pesticides affect the immune system of fish (Areechon and Plumb, 1990).

In conclusion the ELISA used in this study was able to detect changes in the pattern of IgM secretion of the fresh water fish rainbow trout. Accordingly, the modulatory effect of the used chemical stressors, especially CuSO4, could be life threatening to fish. A different local fish modell might contribute effectively, with the present data to the problem formulation and ecological effects evaluation in Egypt.

ACKNOWLEDGEMENT

We thank Dr Norman Miller (Mississippi University, USA) for the gift of mouse anti-rainbow trout IgM monoclonal antibody.

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Table 1: **Absorbance values of rainbow trout IgM and their corresponding reactive titers in correlation with $CuSO_4$ treatmernt.**

Time Laps[#] (weeks)		IgM Abs ± SE (Titer x10^3)			
		Negative Control Fish	Positive Control Fish	Test Fish (CuSO4 Treated) low	high
0	**1st**	0.020 ± 0.003 (3.2)	0.020 ± 0.003 (3.2)	0.020 ± 0.003* (3.2)	0.020 ± 0.003 (3.2)
1		0.030 ± 0.003 (3.2)	0.030 ± 0.005 (3.2)	0.001 ± 0.001* (1.6)	0.040 ± 0.003 (6.4)
2		0.010 ± 0.005 (3.2)	0.010 ± 0.001 (3.2)	0.040 ± 0.010 (6.4)	0.120 ± 0.008* (12.8)
3	**2nd**	0.020 ± 0.003 (1.6)	0.180 ± 0.010* (12.8)	0.150 ± 0.010* (6.4)	0.250 ± 0.010** (25.6)
4		0.020 ± 0.003 (1.6)	0.080 ± 0.010 (6.4)	0.070 ± 0.010 (6.4)	0.140 ± 0.010* (25.6)
5		0.030 ± 0.003 (0.8)	0.110 ± 0.010 (1.6)	0.070 ± 0.003** (25.6)	1.500 ± 0.003** (51.2)
6		0.020 ± 0.003 (0.8)	0.080 ± 0.010* (6.4)	0.900 ± 0.020** (25.6)	1.600 ± 0.030** (51.2)
7		0.020 ± 0.003 (0.8)	0.160 ± 0.010* (6.4)	0.080 ± 0.010 (3.2)	1.600 ± 0.003** (102.4)

Time laps refers to the elapsed time after the beginning of the experiment in none immunized fish or after the 1st immunization in the test fish. 1st and 2nd indicates immunization doses.

Significant at P<0.05 and ** significant at P<0.01, otherwise are none * significant, as compared to the corresponding absorbance values of negative control fish.

Table 2: Absorbance values of rainbow trout IgM and their corresponding reactive titers in correlation with ML treatmernt.

Time Laps[#] (weeks)		IgM Abs ± SE (Titer x10^3)			
		Negative Control Fish	Positive Control Fish	Test Fish (ML Treated) low	high
0	1st	0.020 ± 0.003 (3.2)	0.020 ± 0.003 (3.2)	0.020 ± 0.003* (3.2)	0.020 ± 0.000 (3.2)
1		0.030 ± 0.003 (3.2)	0.030 ± 0.005 (3.2)	0.001 ± 0.001* (1.6)	0.040 ± 0.003 (6.4)
2		0.010 ± 0.005 (3.2)	0.010 ± 0.001 (3.2)	0.030 ± 0.003 (6.4)	0.020 ± 0.003 (6.4)
3	2nd	0.020 ± 0.003 (1.6)	0.180 ± 0.010* (12.8)	0.140 ± 0.010 (12.8)	0.600 ± 0.070** (51.2)
4		0.020 ± 0.003 (1.6)	0.080 ± 0.010 (6.4)	0.050 ± 0.008 (6.4)	0.070 ± 0.008 (6.4)
5		0.030 ± 0.003 (0.8)	0.110 ± 0.010 (1.6)	0.100 ± 0.020 (6.4)	0.400 ± 0.090* (12.8)
6		0.020 ± 0.003 (0.8)	0.080 ± 0.010* (6.4)	3.300 ± 0.100 (102.4)	5.100 ± 0.200** (204.8)
7		0.020 ± 0.003 (0.8)	0.160 ± 0.010* (6.4)	0.800 ± 0.200 (25.6)	2.300 ± 0.100** (102.4)

Time laps refers to the elapsed time after the beginning of the experiment in none immunized fish or after the 1st immunization in the test fish. 1st and 2nd indicates immunization doses.

* Significant at $P<0.05$ and ** significant at $P<0.01$, otherwise are none significant, as compared to the corresponding absorbance values of negative control fish.

Table 3: Absorbance values of rainbow trout IgM and their corresponding reactive titers in correlation with PQ treatmernt.

Time Laps* (weeks)		IgM Abs ± SE (Titer x10^3)			
		Negative Control Fish	Positive Control Fish	Test Fish (PQ Treated) low	Test Fish (PQ Treated) high
0	1st	0.020 ± 0.003 (3.2)	0.020 ± 0.003 (3.2)	0.020 ± 0.003 (3.2)	0.020 ± 0.000 (32)
1		0.030 ± 0.003 (3.2)	0.030 ± 0.005 (3.2)	0.020 ± 0.003 (6.4)	0.080 ± 0.005* (12.8)
2		0.010 ± 0.005 (3.2)	0.010 ± 0.001 (3.2)	0.130 ± 0.010* (12.8)	0.210 ± 0.030* (12.8)
3	2nd	0.020 ± 0.003 (1.6)	0.180 ± 0.010* (12.8)	0.300 ± 0.030* (12.8)	0.370 ± 0.040* (12.8)
4		0.020 ± 0.003 (1.6)	0.080 ± 0.010 (6.4)	0.340 ± 0.020* (25.6)	0.700 ± 0.090* (51.2)
5		0.030 ± 0.003 (0.8)	0.110 ± 0.010 (1.6)	0.020 ± 0.003 (3.2)	0.100 ± 0.009* (6.4)
6		0.020 ± 0.003 (0.8)	0.080 ± 0.010* (6.4)	1.800 ± 0.003** (51.2)	2.300 ± 0.003** (102.4)
7		0.020 ± 0.003 (0.8)	0.160 ± 0.010* (6.4)	0.280 ± 0.040 (12.8)	0.160 ± 0.020* (25.6)

Time laps refers to the elapsed time after the beginning of the experiment in none immunized fish or after the 1st immunization in the test fish. 1st and 2nd indicates immunization doses.

* Significant at P<0.05 and ** significant at P<0.01, otherwise are none significant, as compared to the corresponding absorbance values of negative control fish.

Table 4: Evaluation parameters in relation to fish conditions.

Fish Groups	Evaluation Parameter of Titers			
	Min ($x10^3$)	**Max ($x10^3$)**	**Average ($x10^3$)**	**Doubling* (folds)**
Negative control	0.8	3.2	1.9	1.0
Positive control	1.6	12.8	5.4	2.8
$CuSO_4$ treated				
low	1.6	25.6	9.8	5.16
high	3.2	102.4	34.8	18.3
ML treated				
low	1.6	102.4	20.6	10.8
high	3.2	204.8	49.2	25.9
PQ treated				
low	3.2	51.2	16.0	26.9
high	3.2	102.4	28.4	14.9

* Doubling refers to the number of folds of the titer average values compared to the average titer of negative control fish.

ILLUSTRATIONS

Figure 1: The IgM titers of rainbow trout fish in correlation with immunization with KLH. Negative control (dark shaded bars) represents the IgM pattern obtained from nonimmunized fish. Positive control (light shaded area) represents the normal IgM pattern of immunized fish. The trends of the two patterns were expressed as dotted line (negative control fish) and dashed line (positive fish).

Figure 2: In addition to IgM patterns of negative and positive contol fish shows IgM patterns o ٢figure ،(١expressed as in figure)f fish exposed to low and high concentrations (⅄ and o marked curves, respectivly) of $CuSO_4$ (2a), ML (2b) and PQ (2c). The trends of all patterns were expressed as lines.

Figure 1:

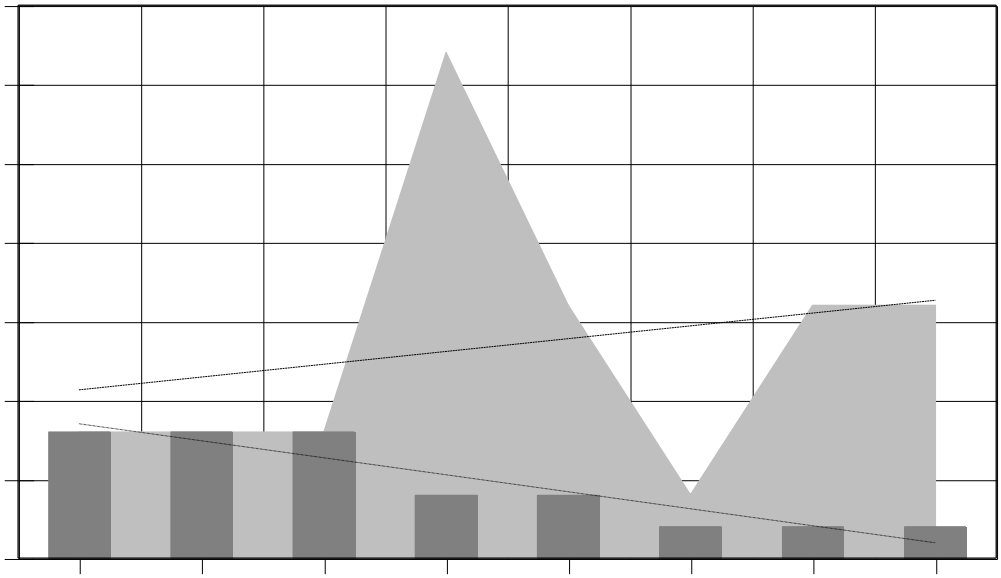


Figure 2a:

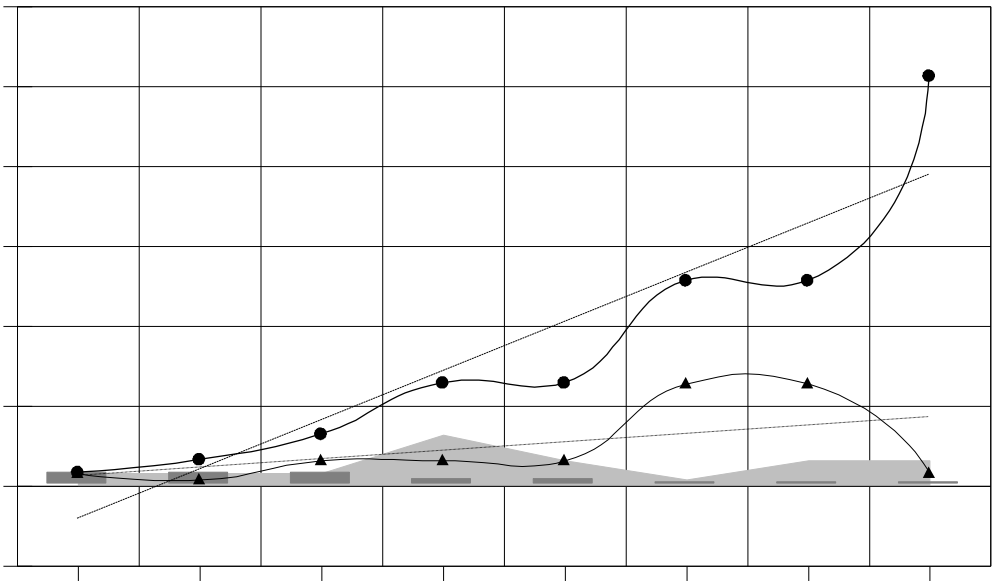


Figure 2b:

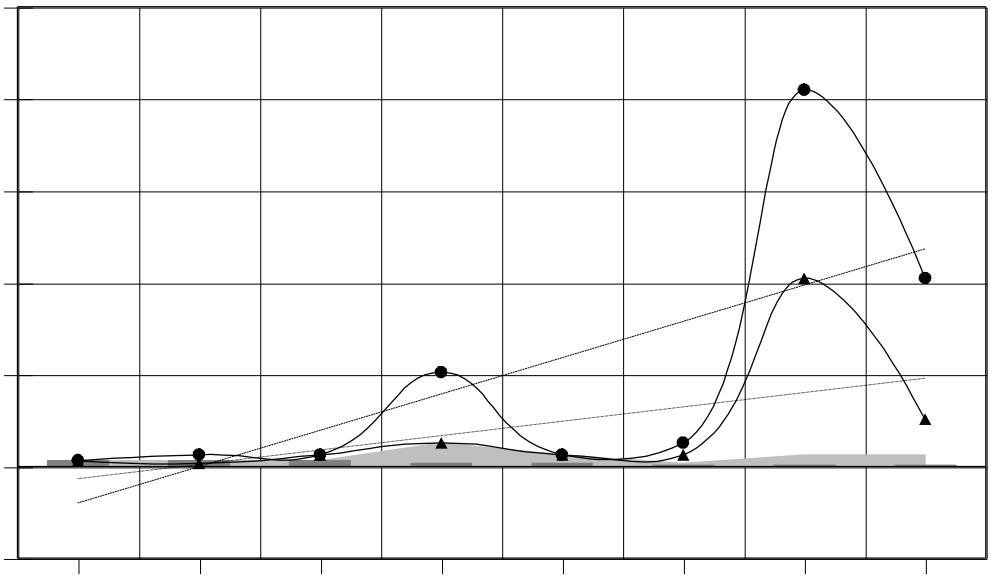
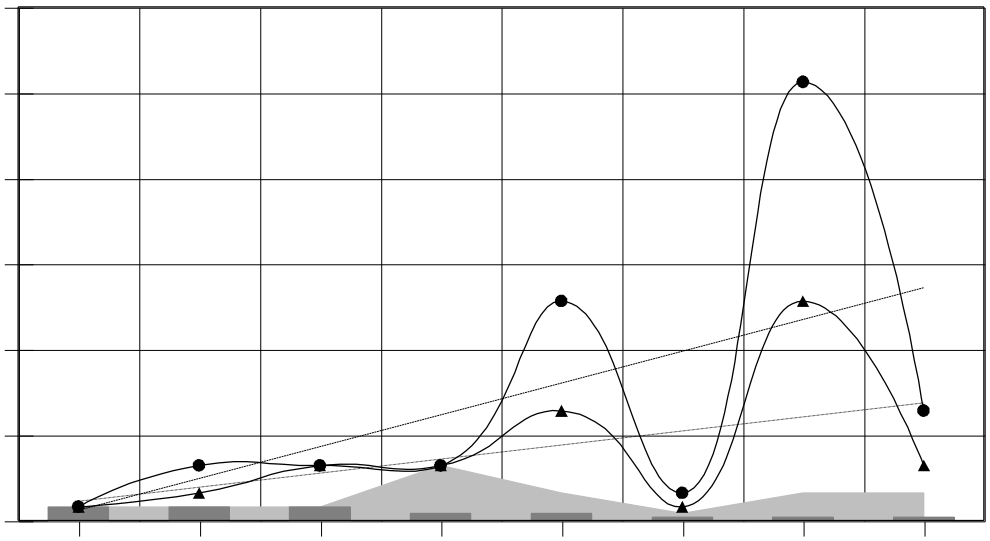


Figure 2c:



MODULATION OF THE HUMORAL IMMUNE RESPONSE OF RAINBOW TROUT EXPOSED TO ECOTOXIC STRESS FACTORS

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ABSTRACT

Unacceptable risks are posed to ecological receptors from chemicals as well as many other pollutants. Chemical contamination is considered a major ecological stress factor on fresh water fish. Stress can cause a reduction in feed intake, impair growth, reproduction and immunity. Copper sulfate, Malathion and Paraquat, as industrial overuse and agricultural pesticides, can cause extensive contamination to aquatic environments. This study is concerned with the determination of the modulating effect of acute sublethal doses of the three chemicals in the fresh water fish rainbow trout (*Onchorynchus mykiss*). A monoclonal antibody dependent indirect enzyme linked immunosorbant assay (ELIZA) was used. The evaluation of the immune modulation was based on the determination the IgM titers in correlation to KLH immunization. Significant changes in the IgM patterns indicated suppression and/or enhancement of the immune system of fish. Such data could be of value for preliminary problem formulation and ecological effects evaluation in ecosystems.

تحور الاستجابة المناعية المصلية للرانبو تروت المعرض لعوامل بيئية سامة

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ملخص

تحمل الكيماويات مثلها مثل كثير من الملوثات الأخرى أخطارا للمستقبلات البيئية. و يعد التلوث الكيميائي عاملا ضاغطا بيئيا عظيم التأثير على اسماك المياه العذبة. وهذا الضغط قد يسبب نقصا في القدرة على الاغتذاء، اختلالا في النمو والتكاثر و المناعة. وقد يسبب تزايد استخدام كبريتات النحاس، الملاثيون و الباراكوات صناعيا أو زراعيا كمكافحات للآفات تلوثا للبيئات المائية. لذلك، تناولت هذه الدراسة التأثير المحور لجرعات تحت مميتة من المواد الثلاثة على سمكة المياه العذبة رانبو تروت. استخدم اختبار الادمصاص المناعى غير المباشر و المعتمد على أحد الأجسام المضادة وحيدة النسيلة. واعتمد تقييم التحوير المناعى على قياس مستويات الأجسام المضادة "م" ارتباطا بتمنيعها بمادة "ك ل هـ". بينت الأنماط المتميزة للأجسام المضادة "م" تثبيط و/أو تنشيط للجهاز المناعى مما دل على ارتباك رد الفعل المناعى. مثل هذه النتائج من الممكن أن تكون ذات فائدة فى تحديد المشكلة و تقييم المؤثرات البيئية فى النظم البيئية المائية.