Practical No: 1.

LC50 Calculation
Predict Toxicity

LC50:

→ The concentration of substances that is lethal to 50% of organism exposed to it in a toxicity test is a useful to all because it can predict the effects of potential in aquaculture system.

→ LC_{50} data can also help to define maximum allowable toxicant. Aquaculturists sometimes are confronted with toxicity of natural or man made substances to fish, shurmps or other culture species.

→ Naturally occuring toxicity can result from dissolved oxygen conc ∝, high conc. of NH_3, CO_2, NO_2 or H_2S.

→ Toxicity also can result from the concentration of culture system of pesticides, heavy metals or industrial chemicals.

Lethal Concentration:

→ The potential toxicity of chemicals substances after is pretended as LC_{50}.

→ LC_{50} can be determined for any exposure time but the most expensive period is 72 hours.

→ If the exposure is longer than the LC50 will be longer.

→ If the exposure is long enough can asymptotic LC_{50} value can be obtained.

→ The asymptotic LC50 is not the time dependent.

Toxicity testing:

Toxicity testing usually are conducted in a glass container in which organism are exposed to the chemicals & their survival rate is checked.

Procedure:

- Acclimilation of test organism should be done for toxicity testing.
- Mortality must be checked frequently & dead organism should be removed.
- The mortality % age should be plotted on semi-long paper versus the toxicant conc. & the conc. that killed 50% of test organism is calculated graphically or by statistical techniques.

Variability:

- There can be great variation in LC50 among toxicity test conducted in different laboratories. e.g the toxicity of substance will increase with increasing of temperature.
- Water quality varibles such as PH or total alkinity also have longer effect on toxicity.
- The concentration of total ammonicum nitrogen necessary to kill 50% of test organism is greater at PH of 7 or below than at at higher PH.

Aquaculture Uses:

- In evaluating the causes of mortality of aquaculture organisms one may find in scientific literature a range of LC50 values for particular toxicant.
- The LC50 value most appropriate for reference in which conditions were similar to those of the aquaculture system of the time of mortality.
- The save conc. of the toxicant is called the maximum allowable toxicant conc. or MATC.
- There is much less life cycle toxicant data available to check LC50 for 96 hours.
- Application factors tend to be smaller for high toxic compounds than less toxic ones.
- In aquaculture, applications factors of 0.05 is suitable for natural toxins, while a factor of 0.01 should be used for industrial chemicals or pesticides.

For Example.

In illustrate the use of application factors, suppose the 96 hours.

LC50 to the culture species for non-ionized ammonia (NH_3) for un-ionized is (1.2 mg/L).

The MATC should be 12 mg/L ∧ 0.05

= 0.06 mg/L.

- Thus if NH_3 conc. remain below 0.06 mg/l. The culture species should suffer no adverse effects.
- Ozcourse toxicology might occurs at conc. of NH_3 below 1.2 mg/l for that value represents the conc. necessary to kill half of organism. A much lower conc. could be expected to cause incipient morlality.
- Sometimes it is not possible to find LC50 for desired temperature.
- Toxicology usually doubles with the increase of 10°C temperature.
- Thus if 2 mg/l is the 96 hours LC50 values of a substance at 20°C the corresponding values at 25°C & 30°C as 1.5 mg/l & 1 mg/l respectively.

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$$\text{Application Factor} = \frac{\text{MATC}}{\text{96 hours LC50}}$$

Practical No 02.

Effect of Ethanol concentration on developing chick embryo.

Background information.

Fatal alcohol syndrome (FAS) results from parental alcohol exposure those affected by disease are born with birth defects & development disorders such as mental retardation (Wellen Lane, 2005).

FAS as noticed within 1% of all live births & unlike other diseases. It in 100% preventable (Burd, et al, 2004).

- Ethanol is one of the component found in one within alcohol. It is known as tratogen. A
- teralogen is a agent that can interfare with the normal development of a fetus.
- Ethanol disrupts the normal development of central nervous system. Primarily the substances inhibits glaid cell proliferation that are the cells that makup NS, provide support & nutrition to the nervous system.
- Specifically with FAS the timing of ethanol exposure after determined the limit of damage.
- The most common exposure of ethanol within the CNS is characteristic of growth suppression.

Procedure:

- (The 2 ozone) Two dosen chicken eggs are kept at room temperature for no longer than two days.

- In preparation of ethanol injections, the small end of each egg were cleaned with 70% ethanol.
- Once cleaned, each egg was then labelled with a pencil according to the designated injection group.
- Next a site was made into the air cell of each egg & 1/2 ml of solution was injected into the air cell.
- The composition of each 1/2 ml of solution was based on the designated dosage group.
- The low dose groups solutions was compared of 2 ml of 200 proof ethanol & 48 ml of distilled H_2O.
- After ethanol was placed inside the air cells of each of eggs. The injected site was sealed with paraffin.
- The eggs were then placed in incubator within an incubator egg turn.
- The incubator was kept at 98° F & the eggs were candeled periodically throughout development. When embryos are removed after opening of eggs, the length & width of each embryo's head was collected using a caliper.
- Observations were also made of any noticable morphological abnormalities.

Results.

- Results showed reduction in embryo's weight.
- higher ethanol conc. have shown an increase in growth, suppresser in both broiler & layer chicken strains.
- Also differences of growth suppressions b/w high & low ethanol dosage.
- Groups could not be concluded usually decrease size & abnormal growth was observed.

Conclusion:-

- The graph shows some decrease in embryonic weights due to ethanol exposure.
- A problem likely exists in the no. of chicken embryo's used within the experiment.
- A large sample size might have demonstrated embryonically growth suppression.
- Additionally the measurment of (mem) embryos head were variable.
- Again the hard caliber data might have been significant if a larger sample size was taken.

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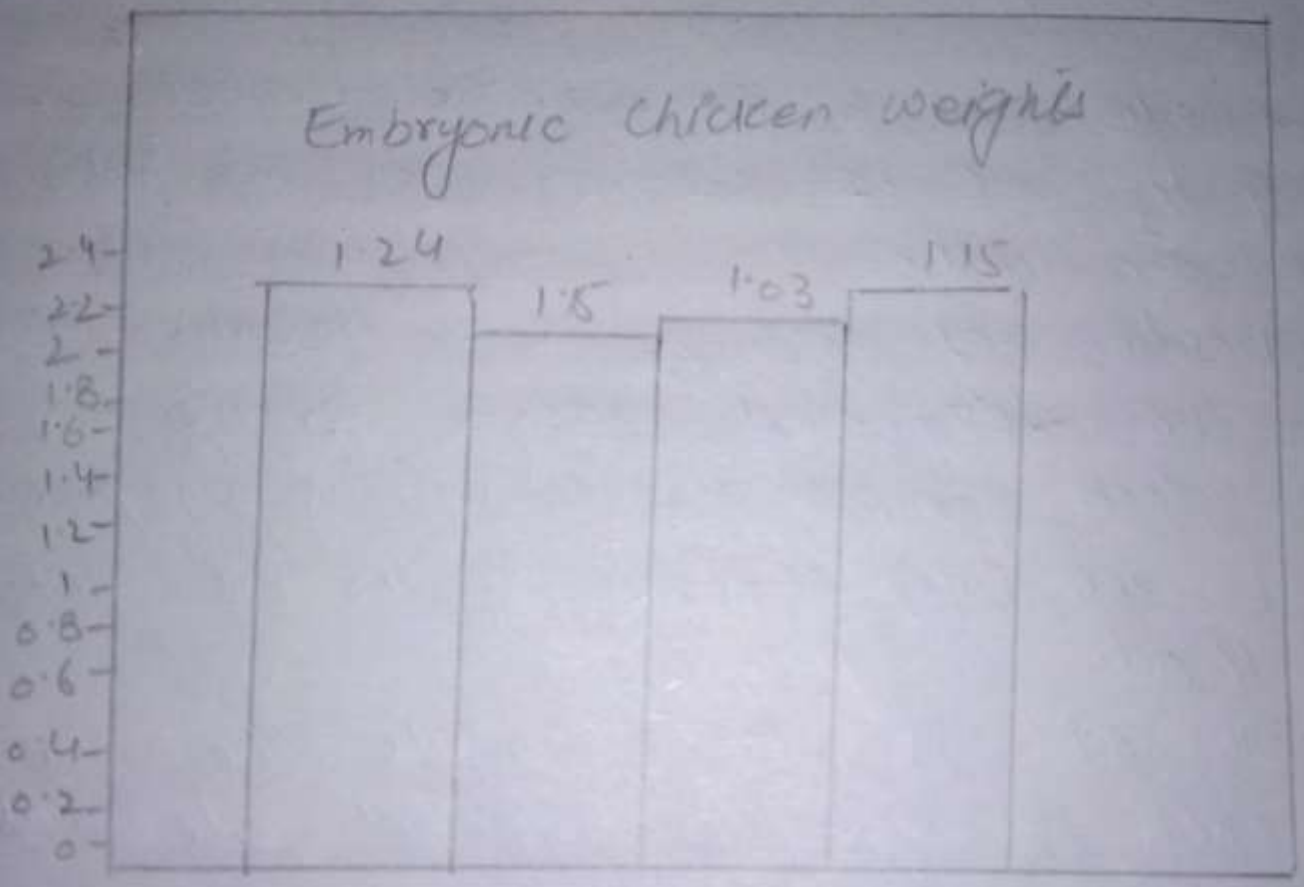
Embryonic chicken weights
1·24
1·5
1·03
1·15
2·4
2·2
2
1·8
1·6
1·4
1·2
1
0·8
0·6
0·4
0·2
0

Practical No 03.

Study of 96 hours LC50 of selected metal on Fish.

Apparatus:

- LC50
- Selected metal
- photo light
- Chromium
- Sedimental tanks
- Aeration
- Air stone
- Pipes
- Aquarium

Procedure:

The fingerlings of Tilapia were taken from experimental ponds & kept in sedimental tanks & acclimitized for 40 days before conducting acute toxicity test.

Preparation of stock Solution:

Pure compound of selected metal was dissolved in deionized water & stock of 1000 ppm was prepared.

Experimental Condition:.

→ Acute toxicity test was performed in glass aquarium of 70 h water capacity.

Metal free tap water was filled in aquarium supplied with aeration system 3 replication were used, 10 individuals of known weighted, length were placed in each aquarium

seperately.

For experimental desired some of metal from stock solution was added in aquarium.

To avoid stress to the fish, the desired metal conc. in each aquarium was attained with in fifteen of 7 hours of stock experiment.

Physical & Chemical Parameter Managment.

→ Temparature, PH, hardness, dissolved O_2, NH_3 also were maintained.

→ To maintain temperature put thermometer & to trails twice a day.

→ During trail fish were subjected To 12 hours photoperiod.

→ Fish were not fed during experimental period.

→ The aquarium were maintained three times a day.

→ The experiment was run with different conc. starting from upper till the lethal conc. was obtained.

→ Each conc. was determined separately & morlality data was recorded.

→ The dead fish obtained during each trail were removed from the tanks and the counted.

→ The arithemetic mean of each trail calculated & expressed in results.

→ Probit analysis mean of each trial was done against fish responded.
By using standard protocole.

Reference:

→ The average of all physical & chemical parameters were subjected through one way of analysis of variance (ANOVA).
(Sled-et-al; 1996)

→ Duncan's multiple range test were used for comparison of mean.

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Practical No. 04

Studies on sublethal concentration of selected metal to check growth response of fish apparatus.

Procedure:

→ The present investigation was conducted at fish hatchery of UVAS.

→ Juviniles of Tilapia were obtained in nursery ponds. Kept in holding tanks & supplied with flow through aerated water.

→ The fish were acclimilized for 3 days before & conducting toxicity test.

10 individuals of similar weight & size of Tilapia were grown in glass aquarium for 3 months under sublethal concentration ($1/3$ of LC50) of given metal with controlled laboratory conditions.

→ Aquaria were filled with 70 liter tap water Temp & PH were monitored regularly. Each aquarium was exposed of sublethal conc. of given metal as determined during 96 hours LC50 experiment.

→ The controlled fish were grown in metal free H_2O. All experimental Fish were fed at rate of 2% of body weight at 9 am & 5 pm daily with 30% digestible protein.

Following feed formation was used 50% Fish meal etc.

Feed Formulation:-

- 50% fish meal
- 47% soya bean meal
- 30% maize gluten
- 12% Rice polish
- 15% wheat bran
- 1% vitamin + mineral

→ The growth trails were performed with 3 replicates. Following parameters were to noted.

Increase / decrease in average net weight.

Increase / decrease in total length, condition factor, feed intake.

→ FCR were monitored weekly.

→ At the end of trial, fish were sacrificed at dissected. Liver, kidney, muscles & fins were isolated & digested to determine the metal conc. through atomic absorption photometer.

→ The data obtained from experiment were statistically analyzed using "SAS".

→ Analysis of variance & DMR were used to find out statistical different regarding growth performance & bioaccumulation in different body organ.

Fish Meal = 100 kg

cp = 35%

Fish meal 20% = 20 kg cp

Rice meal 25% = 25 kg 60%

cotton seed 10% = 10 kg 36%

Maize gluten 10% = 55 kg 41%

Fish meal = $\frac{20}{100} \times 60 = 12$ kg

cotton seed = $\frac{10}{100} \times 41 = 45$ kg

Total Fish meal = 100 kg

= 100 - 55 = 45 kg

cp = 35% - 25% = 10%

Principle of atomic Absorption Photometer:

It is a procedure for the Quantitative determination of chemical element using the absorption of optical radiation by free atom in gaseous state.

Analytical Technique:-

→ Atomic absorption photometery was 1st used as a technique & the underlying principle were established in 2nd half of 19th century.

→ The technique mates use of absorption spectrometery to asses the conc of an analyte in a sample.

→ The atomizers most commonly used in AAS are flames & graphite tube atomizer. Other autonizer are glow discharge atomizer, hydride atomizer or vapour autonizers might be used for special purpose.

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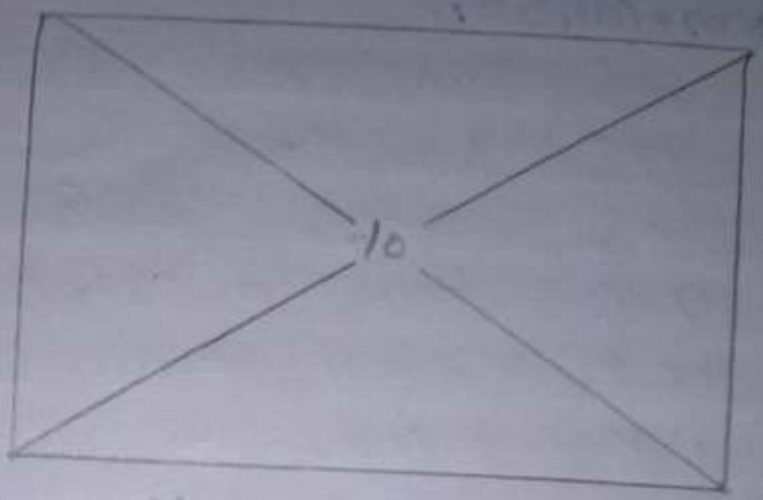
Rice polish
12%



20 kg

Maize gluten 30%

2kg

Rice polish = 22 kg

Maize gluten = 20 kg

Rice Polish = $\frac{2}{22} \times 45 = 4.09$

Maize gluten = $\frac{20}{22} \times 45 = 40.90$

= 45 kg

Practical No: 05

Bioaccumulation pattern of selected metal in Fish.

Apparatus:

- Animal
- Selected metal (Nickel)
- Small plastic
- Photospectrometer
- Bag
- Fish
- Atomic Absorption Photometer

Bioaccumulation:

Paper to the accumulation of substances such as pesticides or other chemical in an organism.

Digestion:

Bioaccumulation occur when an absorbs a toxic substance at a rate greater than that at which the substance is lost.

There are two types of digestion are wet & other are the digestion. Comparison of wet & dry digestion.

Dry digestion:

The dry & wet digestion methods of processing food samples for elemental analysis. The conc. of trace element (mg, Fe, Cu, Zn)

Chromium calcium

Lead nickle

were determined in varieties of samples. The metal conc were determined using atomic absorption photometer according to standard method.

Wet digestion.

The accuracy of the procedure was confirmed by spiking some samples and evaluting their recoverives.

The metal levels evaluated were relatively higher in the dry ashed samples than the wet.

Biomagnification:

Biomagnification is also known as (biomani) bioamplification or biological magnification occur. When the conc. of a substances such as PPT or mercury in an organism exceeds. The background conc. of the substances in its diets.

Organs in which bioacclimulation Determine:

- liver
- Kidney
- Gills
- Gut
- Muscles
- Skin
- Scale
- Fins

Probit Analysis.

Probit analysis is a type of regression used to analyzed binomial response variable. (A) It transform the signoid dose = Response curve to straight line that can then be regression either through least square or maximum hood.

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Practical No. 06

Preparation of toxic solution of heavy metals.

Preparation of toxic soln of heavy metal for 50 L (5000 ml).

Iron Sulphate $Fe(SO_4)_3$ Nickle Sulphate $NiSO_4$

Fe = 55.48 mol wt.

mol wt of $NiSO_4$ = 154.75 gm/mol.

1000 ppm = 1000 mg/L ⇒ 1gm/L.

$$Ni = \frac{154.75}{58.69} = 2.0 \text{ gm}$$

Stock soln = 2 gm of $NiSO_4$ were dissolved in 1000 ml/L that is stock soln.

$$C_1V_1 = C_2V_2$$

$$1000 \times x = 2 \times 50000$$

$$x = 100 \text{ ml}$$