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Utilization of lime sludge waste from paper mills for fish culture

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Lime sludge (mud) waste from a paper mill at Jagiroad, Assam (Hindustan Paper Corporation Limited) can be used as a cheaper alternative source of liming for management of acidic water in the fish ponds of Assam. Lime sludge waste has no toxic substances, but contains 66.5% calcium carbonate. To maintain the alkalinity level of water, 1.5 times more lime sludge waste is required compared to pure lime. Parameters like pH, conductivity, free carbon dioxide, total hardness and dissolved oxygen are increased due to application of lime sludge waste from the paper mill, which are beneficial to the fish as well as fish food organisms. The heavy metals found in the lime sludge waste were below permissible level. Lime sludge has no adverse impact on growth and development of fish, rather it has some beneficial effect on production of fish.

Keywords: Fish production, lime sludge, paper mill, pond management.

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ASSAM, situated in the northeastern region of the country, is endowed with vast and varied fishery resources in the form of ponds and tanks (2.5 m ha), floodplain wetlands (*beels*) and swamps (11.2 m ha), and the rivers Brahmaputra and Barak with their numerous tributaries (combined length 4820 km) covering about 334.5 m ha[1]. However, it is stated that the growth and development of fishes are not up to the mark due to acidic nature of water in most of the fisheries of the state. Therefore, it is difficult to achieve the target of fish production. Phytoplankton growth in acidic waters is often limited by inadequate carbon dioxide and bicarbonate ions. Some waters are so acidic that fishes do not survive or grow well. Muds in ponds also are acidic and strongly adsorb the phosphorus added in the fertilizer[2]. Soil pH in the range of 6.5 to 7.5 and water pH in the range of 6.5 to 9.0 are considered to be optimal for fish production[2]. Soils of the state are acidic[3], ranging from 4.6 to 6.8, which is below the optimal range. Thus, in order to achieve optimal fish production, the fish ponds of the state need adequate liming. Lime increases the pH of bottom muds and makes phosphorus readily available, besides increasing the pH and total hardness of pond water. However, addition of large quantities of lime increases the cost of fish production. For example, for correcting acidity of soil in the range of 6.0 to 6.5, a dose of 1000 kg/ha/yr of quick lime (CaO) is required[4], costing an additional financial burden of Rs 6000 (at current prices). Thus, application of lime in fish ponds becomes a costly but unavoidable management option for the poor and marginal fish farmers of the state. Therefore, it has become imperative to search for a cheaper alternative to pure lime for application in fish culture ponds. Limes sludge waste from the Hindustan Paper Corporation Limited (HPC) at Jagiroad, Morigaon district, Central Assam, can be used as alternative source of liming, as it contains high amount of lime[5].

The paper mill generates large quantities of lime sludge every day (940 tons/day). Disposal of this solid waste has become a problem, as it causes abnormal increase in soil alkalinity and damage to vegetation in the vicinity of the dumping ground. Utilization of this waste for fish culture will hopefully serve the dual purpose of pollution abatement as well as reducing the cost of fish culture operation in the state. However, before applying the waste into the fish ponds, it should be ascertained if it has any substance which is toxic to fishes or fish food organisms. Against this background, the present investigation has been carried out for extensive field and laboratory studies on utilization of lime sludge waste from the paper mill for fish culture.

The lime sludge waste was collected in a gunny bag from the dumpsite of the paper mill. The collected waste was first dried in the laboratory and crushed into fine powder for analysis of heavy metals (Pb, Zn, Cu, Hg). For metal determination, the lime sludge is dried and then powdered. The powdered lime sludge is then digested in a microwave digester using nitric acid and hydrogen fluoride. Shimadzu AAS 680 Atomic Absorption Spectrophotometer determined the concentration of Pb, Zn, Cu and Hg by atomizing aqueous samples in air acetylene flame. Hollow cathode lamps of individual metals were used to produce respective resonance lines.

Lime sludge waste was dissolved in different concentrations in a beaker to determine the required dose to maintain an alkalinity within a desirable range. Pure lime was also dissolved in another beaker simultaneously.

Three aquaria each containing 65 l of tap water were taken to study the effect of lime sludge waste and pure lime on water quality and fish growth. The initial pH of the water was as follows: aquarium 1 (lime sludge waste), pH 7.25; aquarium 2 (pure lime), pH 7.26 and aquarium 3 (control), pH 7.00.

The marketed lime and lime sludge waste were dried and powdered. They were then added in small beakers in different concentrations to standardize the doses to maintain the pH of water in the aquariums.

In aquarium 1, 22.5 g of lime sludge waste was added to maintain the pH in the range of 7 to 8. In aquarium 2, 15 g of pure lime was added to maintain the same pH.

Aquarium 3, was kept as control without application of sludge and pure lime. After addition of lime sludge and pure lime, the pH and conductance of the aquaria were as follows: aquarium 1, pH 8.20 and conductance (mS/cm) 0.836; aquarium 2, pH 8.10, conductance 0.836; and aquarium 3, pH 8.13, conductance 1.022.

Fingerlings of Indian major carp (Rohu) *Labeo rohita* ranging from 4.5 to 6.5 cm in length and 9 to 11 g weight were collected from local freshwater resources and acclimatized for 1 month to recover from stress and fed with fishmeal in well-aerated water. In each aquarium, 10 fishes of the same size and weight were introduced. They were fed with fishmeal everyday, 0.1 g/fish. Doses of fishmeal were gradually increased. Certain physico-chemical parameters of the aquarium have been analysed at an interval of 30 days for 6 months.

To study the toxicity of lime sludge waste from paper mill on fish, six well-cleaned rectangular glass tanks of 10 l capacity were taken and lime sludge waste was added into the first five aquaria in different concentrations, viz. 1, 5, 10, 15 and 20%. One aquarium was kept as control. Three fishes were introduced in each aquarium and were cultured for a period of one month under laboratory conditions. Mortality of the fish, if any, was also noted and dead fish were removed immediately during the course of culture practice. The different physico-chemical parameters of the culture water were analysed before addition of the fishes and also at the end of the experiment by standard methods[6,7].

To study the effect of lime sludge waste on water and soil quality parameters and also on the growth and development of fish, two ponds of equal size (25 m × 20 m × 2.5 m) were taken. Lime sludge waste was added in one pond at an optimized dose before the start of the rainy season and one pond was kept as control without applying lime sludge waste. Soil from the two ponds was analysed before starting the experiment by standard methods[7]. After 15 days of addition of lime sludge waste, fingerlings of Indian major carp (rohu, catla, mrigala) were added at the density of 5000/ha in each pond. Fish food (rice bran and mustard oil cake at 1 : 1 ratio) was given daily at a recommended dose[8]. The different physico-chemical parameters of water from the two ponds were determined monthly. The length and weight of each fish were taken at the end of the experiment to determine the length–weight relationship. Statistical relationship between the length and weight of fishes was derived using the formula:

$$W = aL^n,$$

where W is the weight of the fish, a is a constant, L the length of the fish and n an exponent to be calculated empirically.

For practical purpose, this relationship is usually expressed in its logarithmic form[9]:

$$\log w = \log a + n \log L.$$

Physico-chemical characteristics of lime sludge (mud) waste of Jagiroad paper mill (Assam) are presented in Table 1. The pH of the lime sludge waste was 10.98, which

Table 1. Characteristics of sludge waste from Jagiroad paper mill and marketed lime

Parameter (unit)	Lime sludge waste from paper mill	Marketed lime
pH	10.93	11.73
Water-holding capacity (%)	70.43	58.1
Organic carbon (%)	0.12	0.029
Conductivity (mS/cm)	2.86	11.73
Calcium carbonate (%)	66.5	99.0
Chloride (ppm)	560.0	4615.0
Available phosphorus (ppm)	0.078	0.086
Available potassium (ppm)	3.7	109.0
Available calcium (ppm)	494.0	237.0
Available sodium (ppm)	30.0	137.0
Heavy metals (ppm)		
Pb	0.514	0.975
Zn	0.38	0.36
Mn	1.375	4.414
Cu	0.064	0.156
Hg	ND	ND

ND, Not detected.

Table 2. Physico-chemical parameters of aquatic media before introduction of fish

Aquarium	pH	Conductivity (mS/cm)	Free CO_2 (mg/l)	Total hardness (mg/l)	Total alkalinity (mg/l)	Chloride (mg/l)	Dissolved oxygen (mg/l)
1 (Lime sludge waste mixed)	8.24	0.836	2.2	108	175	80.06	5.06
2 (Pure lime mixed)	8.15	0.962	4.4	110	190	85.06	2.86
3 Control	7.29	1.022	4.4	110	190	85.06	2.86

Table 3. Monthly changes in physico-chemical parameters of aquatic media after introduction of fish

Month	Aquarium	pH	Conductivity (ìS/cm)	Free CO_2 (mg/l)	Total hardness (mg/l)	Total alkalinity (mg/l)	Chloride (mg/l)	Dissolved oxygen (mg/l)
First month	1	8.20	0.789	2.38	120	175	80.86	5.88
	2	8.10	0.786	2.38	110	160	78.01	4.86
	3	7.30	0.664	4.75	102	185	82.06	3.04
Second month	1	8.30	0.790	4.93	140	165	83.78	5.88
	2	7.71	0.780	4.75	135	150	79.52	4.26
	3	8.00	0.660	4.68	110	170	69.58	2.88
Third month	1	8.01	0.785	9.93	165	185	75.06	8.60
	2	7.68	0.765	7.68	158	176	70.05	5.88
	3	7.78	0.656	4.75	140	160	68.06	2.88
Fourth month	1	8.03	0.791	12.42	195	195	70.60	9.45
	2	7.76	0.748	7.40	178	165	68.08	5.47
	3	7.65	0.640	4.93	150	110	65.40	2.86
Fifth month	1	7.99	0.780	9.75	180	180	60.50	8.68
	2	7.70	0.730	9.64	150	155	63.60	5.88
	3	7.50	0.645	7.40	140	120	58.70	3.24
Sixth month	1	7.72	0.776	7.68	195	160	51.06	9.32
	2	7.68	0.735	7.40	178	145	54.50	5.47
	3	7.54	0.630	4.93	150	110	70.06	3.28

Aquarium 1, Lime sludge mixed water; Aquarium 2, Pure lime mixed water; Aquarium 3, Control (without lime sludge or pure lime).

is slightly lesser than the pH of the pure lime (11.79). The water-holding capacity of lime sludge waste was 70.43% and that of pure lime was 58%. The percentage of $CaCO_3$ in lime sludge waste was 66.5% and in pure lime 99%. The metals present in lime sludge waste were below toxic level.

Hence the dosage required for lime sludge waste is 1½ time more than that of pure lime or marketed lime.

Results of water quality of the aquaria before addition and after addition of fish are presented in Tables 2 and 3. The pH of the aquatic media increased from 7.29 to 8.24 after addition of lime sludge waste and it was within the desirable range. A pH between 6.5 and 9 is considered as the desirable range for most freshwater fishes[2]. The conductivity ranges of the three aquatic media were 0.776–0.798 mS/cm (lime sludge mixed), 0.735–0.786 mS/cm (pure lime mixed) and 0.630–0.664 mS/cm (control; Table 3). Free carbon dioxide ranged between 2.38 and 12.42 mg/l in the lime sludge mixed water, 2.38 to 9.64 mg/l in pure lime mixed water and 4.75 to 7.40 mg/l in the control, which is within the desirable range. The optional range[8] of free CO_2 is 5 mg/l. Total hardness also increased after addition of lime sludge waste. The range of total hardness is from 120 to 195 mg/l in lime sludge mixed water, 110 to 178 mg/l in pure lime mixed water, and 102 to 150 mg/l in the control. Hardness below 20 mg/l is harmful to fish[10]. It was also revealed from the study that the total alkalinity is high in lime sludge waste mixed water. The range of total alkalinity is from 160 to 195 mg/l in lime sludge mixed water, 145 to 160 mg/l in pure lime mixed water and 110 to 185 mg/l in control (i.e. without application of sludge or pure lime). Alkalinity less than 20 mg/l is considered to be harmful for fish farming[2]. Highly productive water has an alkalinity[11] above 100 mg/l. Chlorides in waters are generally due to salts of sodium, potassium and calcium. Chloride ranges from 51.06 to 80.86 mg/l in lime sludge mixed water, 54.50 to 79.52 mg/l in pure lime mixed and 58.70 to 82.06 mg/l in control, which is within desirable range. The higher values of chloride may be due to maximum growth of planktons[12]. Increasing concentration of chlorides is an indication of eutrophy. In the present investigation, dissolved oxygen was found to vary from 5.88 to 4.45 mg/l in lime sludge mixed water, 4.26 to 5.88 mg/l in pure lime mixed water and 2.86 to 3.28 mg/l in control. A concentration of 1.4 mg/l oxygen is sufficient to maintain life in water[13].

After 30 days of exposure of fishes in the lime sludge waste mixed water, there was no toxic effect on the fishes up to 15% concentration; however, at 20% concentration

Table 4. Physico-chemical properties of water from the two ponds during the course of the experiment

Month	Sample	pH	Conductivity (mS/cm)	DO (mg/l)	TA (mg/l)	TH (mg/l)	Free CO_2 (mg/l)
August	Pond with lime sludge	8.44	0.108	6.88	50	22	4.4
	Control (without lime sludge)	8.01	0.065	7.1	20	14	6.6
September	Pond with lime sludge	8.64	0.208	6.5	52	40	4.75
	Control (without lime sludge)	7.95	0.077	6.9	20	10	2.37

Table 5. Physico-chemical properties of soil from the two ponds before and after mixing lime sludge waste

Treatment	Sample	pH	Conductivity (mS/cm)	Water-holding capacity (%)	Total organic carbon (%)
Before application of lime sludge	Pond with lime sludge	6.06	0.149	47.03	0.263
	Control	5.40	0.107	47.09	0.234
At the end of the experiment (after 75 days)	Pond with lime sludge	6.85	0.162	50.25	0.370
	Control	5.55	0.132	47.12	0.280

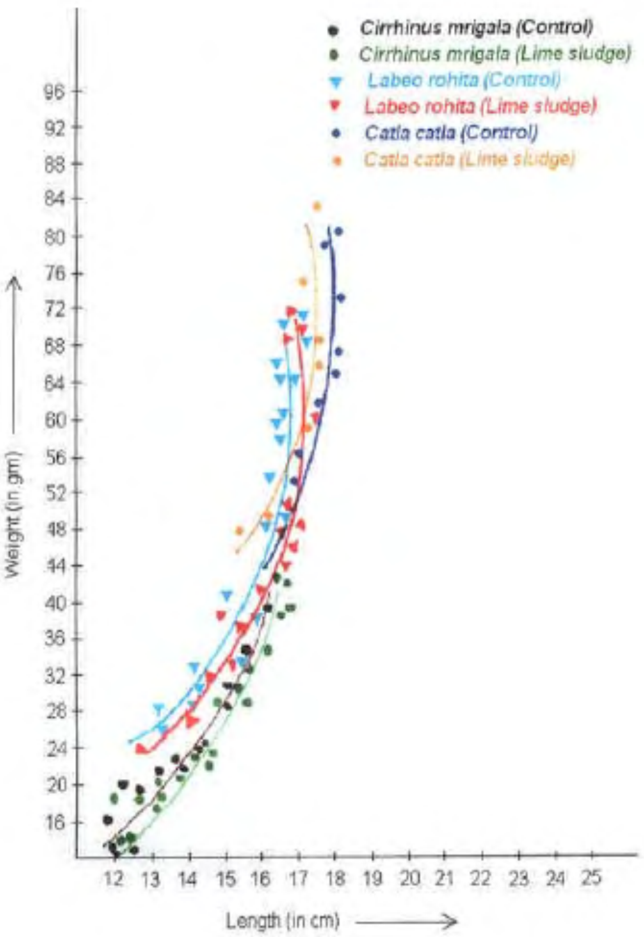

Figure 1. Relationship between length and weight of the cultured fishes (cultured in both line sludge mixed pond and control pond).

mortality occurred. The fish may have died due to turbidity of the water or respiratory problem.

The pH of the water increased after application of lime sludge waste up to 8.64 (Table 4). The conductivity also increased after the addition of lime sludge waste. The total alkalinity also increased from 20 to 52 mg/l; the optimal level is 40–150 mg/l. Alkalinity has direct effect on the production of plankton[8]. It was also revealed from the study that total hardness rises up to 40 mg/l. If hardness of water is less than 11 mg/l, it requires liming[9]. The level of free CO_2 was also within optimal range. The optimal level[8] of free CO_2 is 5 mg/l. Dissolved oxygen varied from 6.5 to 6.88 mg/l in lime sludge mixed water and 6.9 to 7.1 mg/l in control, which were within the desirable level. The optimal range of dissolved oxygen[8] is between 5 and 8 mg/l. It also has a good impact on the soil. The water-holding capacity increased from 47.03 to 50.25% after application of lime sludge waste (Table 5). The pH of the soil also increased after application of lime sludge waste. The observed length–weight relationship of the cultured fishes reared in two ponds is depicted in the Figure 1. The study also revealed that the fishes cultured in lime sludge mixed water showed normal growth. There was no adverse impact of lime sludge waste, affecting the growth of fishes. Thus lime sludge waste can be used as an alternative source of liming in fish farming.

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Use of peripheral blood lymphocyte culture in the karyological analysis of Indian freshwater turtles, *Lissemys punctata* and *Geoclemys hamiltoni*

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A simple, efficient and economic method has been optimized for *in vitro* culture of peripheral blood lymphocytes from two freshwater turtles, *Lissemys punctata* (Trionychidae) and *Geoclemys hamiltoni* (Batagurinae), for various parameters like culture media, mitogen concentration, mitotic index, culture volume, incubation time, humidity, duration of culture or mitotic arrest, etc. The most optimal condition for good mitotic index was obtained when the lymphocytes were cultured at 28°C in a common RPMI-1640 medium with L-glutamine but without $NaHCO_3$, 10% FCS, PHA-P as mitogen (10 μg/ml), streptomycin (100 μg/ml), penicillin (50 μg/ml) and incubated for a period of 84 h. The mitotic arrest was made for 10 to 12 h with colcemid (0.2 μg/ml) after 72 h of setting the culture. The non-banded mitotic chromosomes of both the species were characterized. The diploid chromosome (2N) numbers were found varying in the two species, being 66 in *L. punctata*, and 52 in *G. hamiltoni*. The difference in the chromosome numbers in the two species was basically in the telocentric- and micro-chromosomes, both being higher in *L. punctata* than in *G. hamiltoni*. Except for some of the large or macrochromosomes most others appeared species-specific, indicating their distinct evolutionary lineage among Chelonians.

Keywords: Chelonia, *Geoclemys hamiltoni*, *Lissemys punctata*, lymphocyte culture, turtle karyotype,

FOR studies on cytotaxonomy or chromosomal polymorphisms in endangered wildlife species, the source or number of animals becomes limiting. The classical methods of chromosome preparation from spleen, kidney or intestine[1,2] or cell culture of heart and skin fibroblast[2] involves killing or surgery, often being unsafe for survival of the animal, traumatic and unethical. In such cases, karyotype analysis from cultured blood lymphocytes without sacrificing the animal is most desirable and useful, particularly in reptiles whose body temperature varies with environment and exhibits a slow cell-division cycle. Few reports are available on lymphocyte cultures of reptilian species such as *Alligator mississippensis*[3,4] and *Trachemys scripta*[5]. Not all the cell-culture parameters optimized in the above methods are applicable to Indian species because of their differential adaptability, variation in preferred body temperature, lymphocyte population and immunological responses against mitogen stimulation.

Cytogenetic identification is one of the important parameters for the conservation of organisms in their natural habitat. For this purpose, the first level of genome analysis involves karyotyping of mitotic chromosomes to know the organization of the organismal genome at the cytological level. Turtles and tortoises are the most distinctive reptilians that have retained their basic features since Triassic period. From the viewpoint of cytotaxonomy and molecular cytogenetics, this is the most neglected group of reptiles. Only limited published data are available on the morphology of chromosomes and karyological trends of evolution in turtles[6–9]. The other most significant characteristic of freshwater turtles that tempt detailed cytogenetic study on the mitotic or meiotic chromosomes is their recognition as indicators of water quality of an aquatic body. They can, therefore, be used to assess genotoxicity induced by potential toxicants discharged in the water bodies following cytogenetic analysis of chromosomal aberration[10]. The present report is a successful attempt on an *in vitro* culture of peripheral blood lymphocytes for the preparation of mitotic chromosomes from two species of freshwater turtles, *Lissemys punctata* and *Geoclemys hamiltoni*, for studies on cytotaxonomy and chromosomal polymorphism. The non-banded karyotypes of *L. punctata* and *G. hamiltoni* prepared from short-term lymphocyte cultures revealed species-specific variations in their chromosome complements, mostly in telocentric and morphologically indistinct micro-chromosomes.

Experiments were carried out on young adult individuals of soft-shell turtle *L. punctata*, which were collected

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