Short term effects of crude extracts of cynobacterial blooms of reservoirs in high prevalence area for CKD in Sri Lanka on mice

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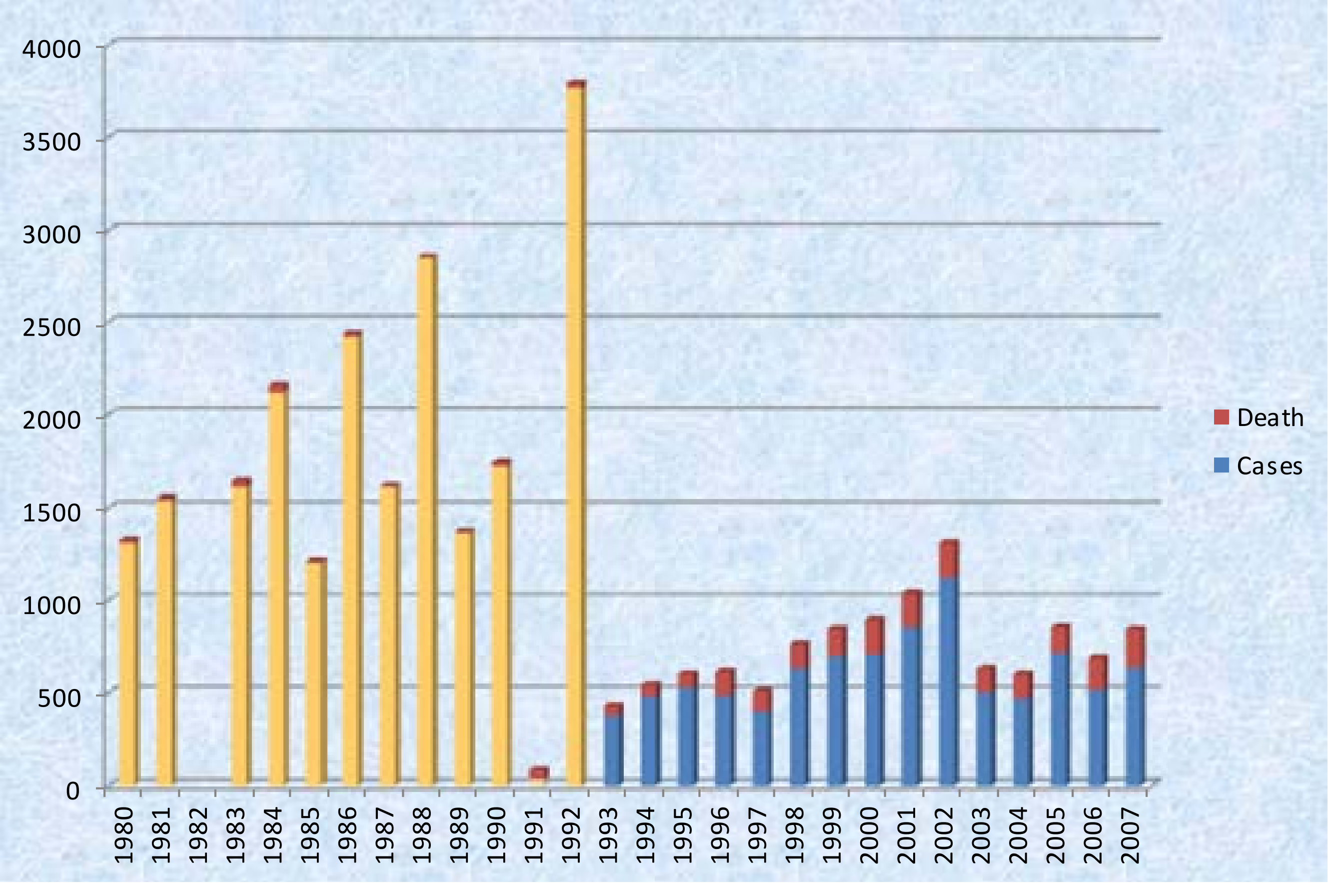
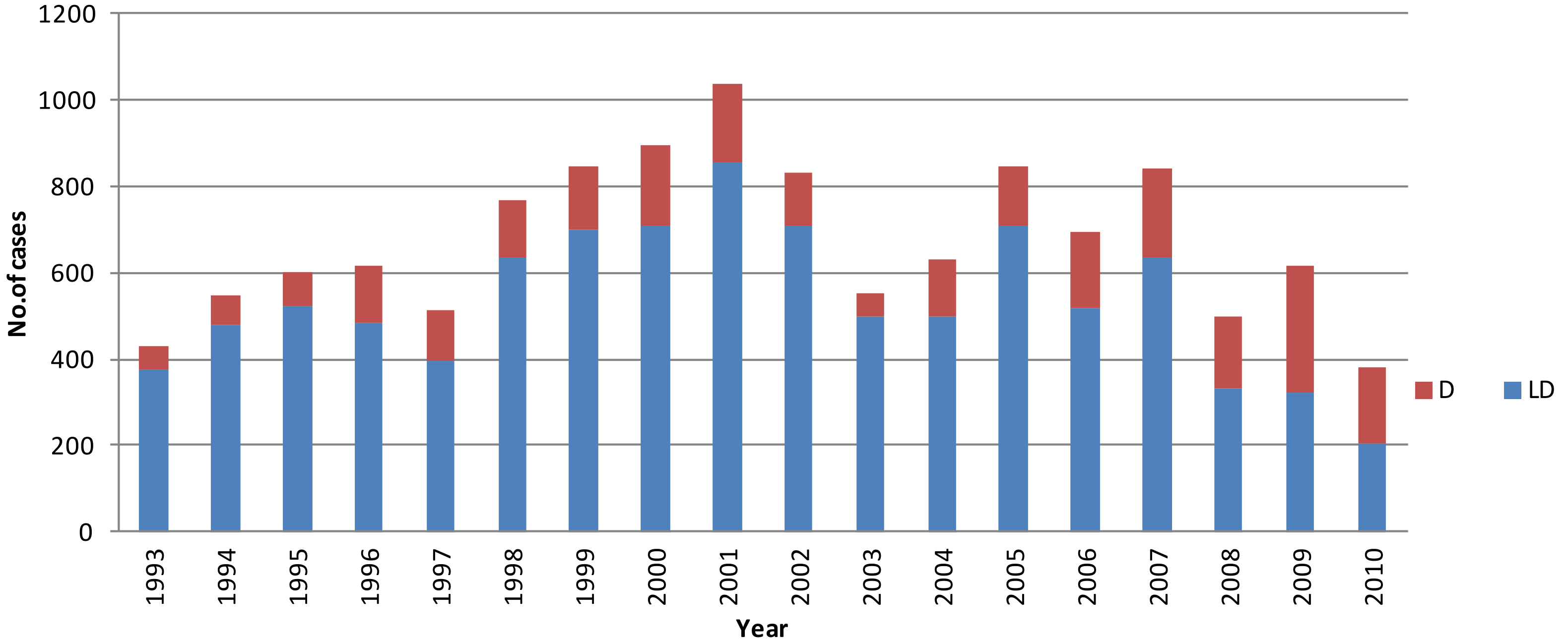
Introduction

- The epidemiology of the chronic kidney disease of unknown origin (CKDu) in Sri Lanka shows distribution of patients around the water reservoirs.
- Histopathology of the renal disease shows evidence of a tubulointerstitial nephritis indicating a possibility of toxic aetiology.
- Similarity in the variations of incidence over time in CKD-U and alcoholic liver disease in the North Central Region indicates the possibility of a toxin with hepatotoxic & nephrotoxic effects.

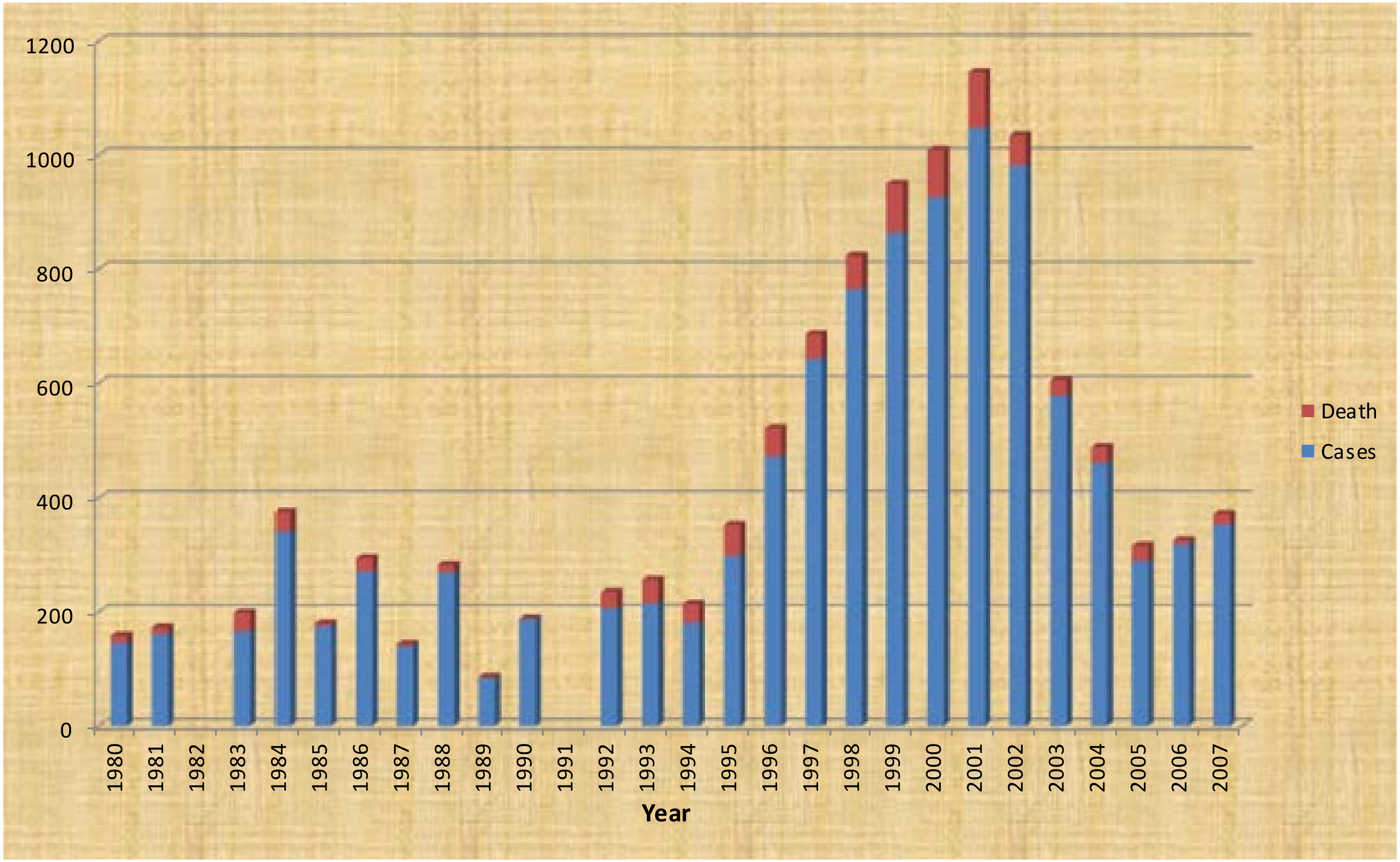
Number of patients with genito urinary diseases presented to G. H. Anuradhapura



Hospitalization-Chronic renal failure from 1993-2010



Number of patients with alcoholic liver disease admitted to G. H. Anuradhapura



Cyanobacterial toxin

- Some cyanobacteria that exist in water reservoirs are capable of secreting toxins in certain adverse environmental conditions.
- Cyanobacterial toxins are known to have hepatotoxic, dermatotoxic, neurotoxic effects in humans and nephrotoxic effects in experimental animals.
- cyanobacterial toxins are highly water soluble & heat stable substances with good stability at room temperature.

Hypothesis

- **The nephrotoxicity and hepatotoxicity could be caused a single agent or by a mixture of substances produced under similar environmental conditions**
- **Cyanobacteria can produce a toxins with nephrotoxic and hepatotoxic effects.**
- **Cyanobacterial toxin is water soluble and heat stable substance.**

To test this hypothesis

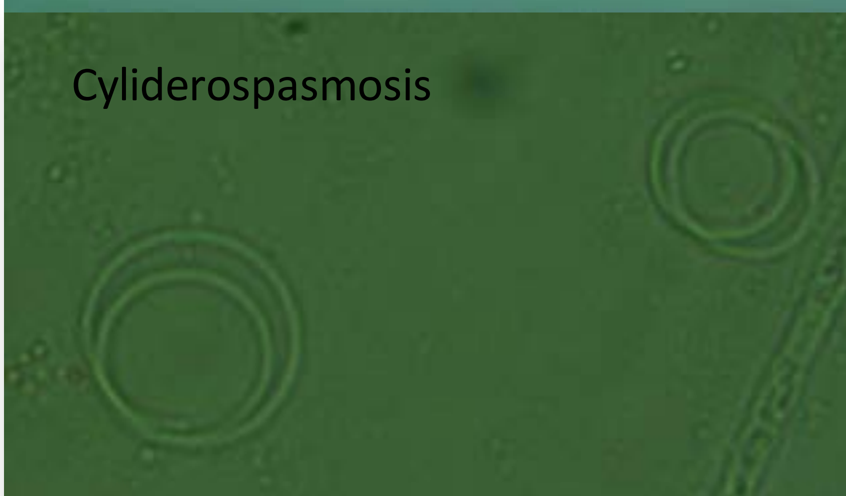
- We should demonstrate that these reservoirs contain toxin producible cyanobacteria
  - Environmental study was planned
- We should demonstrate the presence of cyanobacterial toxin in the crude extracts
  - Crude extracts of cyanobacterial blooms collected from CKDu prevalent area were analyzed
- We should demonstrate that the toxins of these cyanobacteria are capable of producing renal tubular damage
  - Mice were used as the experimental model

- Aim
  - To study the short term effects of the crude extracts of cyanobacterial blooms from the high CKD-U prevalence area on mice kidney.
- Method & material
  - Crude extracts of the cyanobacterial blooms from the reservoirs and canals were prepared (WHO guidelines)
  - Extracts were diluted with distilled water and used for feeding the mice for one week & the control group of mice were fed with water from non CKD-U prevalence area

Cyanobacterial biodiversity in reservoirs and canals of high prevalence area.

- WHO monographs on cyanobacteria describes 18 different types of cyanobacteria capable of producing toxins under favorable conditions
- We have identified 15 toxin producible cyanobacteria in our reservoirs and canals
- Biodiversity of cyanobacteria were studied in selected locations of reservoirs and canals

Toxin producible cyanobacteria in our reservoirs and canals of affected regions

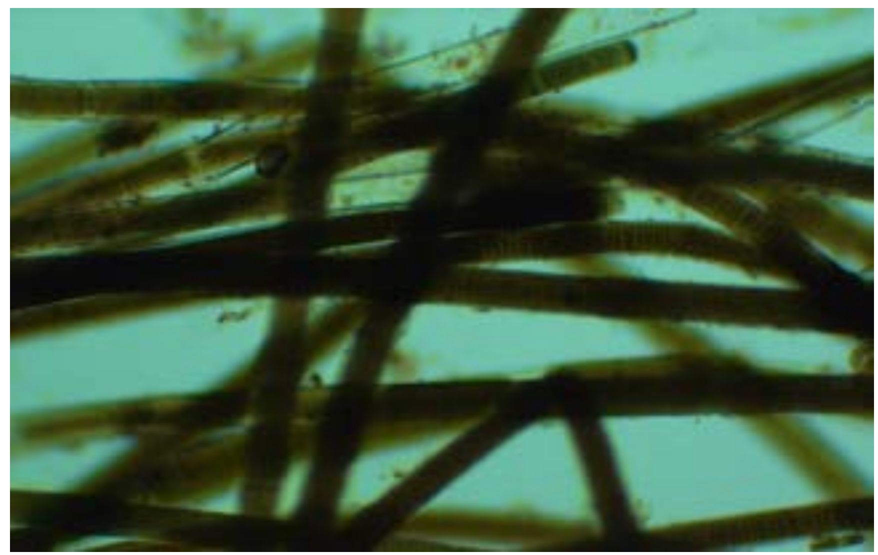


Reservoirs in high prevalent regions show more biodiversity in cyanobacteria

The canals show more diversity of toxin producible cyanobacteria than the reservoirs.

Ulhitiya reservoir



- Cyanobacterial blooms were identified isolated from reservoirs and canal water by filtration
- Crude extracts of cyanobacteria were prepared using WHO guidelines.
- Extracts were used in the
  - Short term toxicity study (1 week exposure)
  - Chemical analysis

Analysis of cyanobacterial toxin

- Samples were analyzed at **National Research Centre for Environmental Toxicology (EnTox) at Queensland** using following methods

- Cyanobacterial toxin
  - **Cylindrospermopsin (CYN)** and
  - **Deoxy Cylindrospermopsin (DCYN)** by LC/MS/MS, reporting limit > 0.2 ug/L
  - **Microcystins**, expressed as total microcystin, done by LC/PDA, reporting limit > 0.5 ug/L

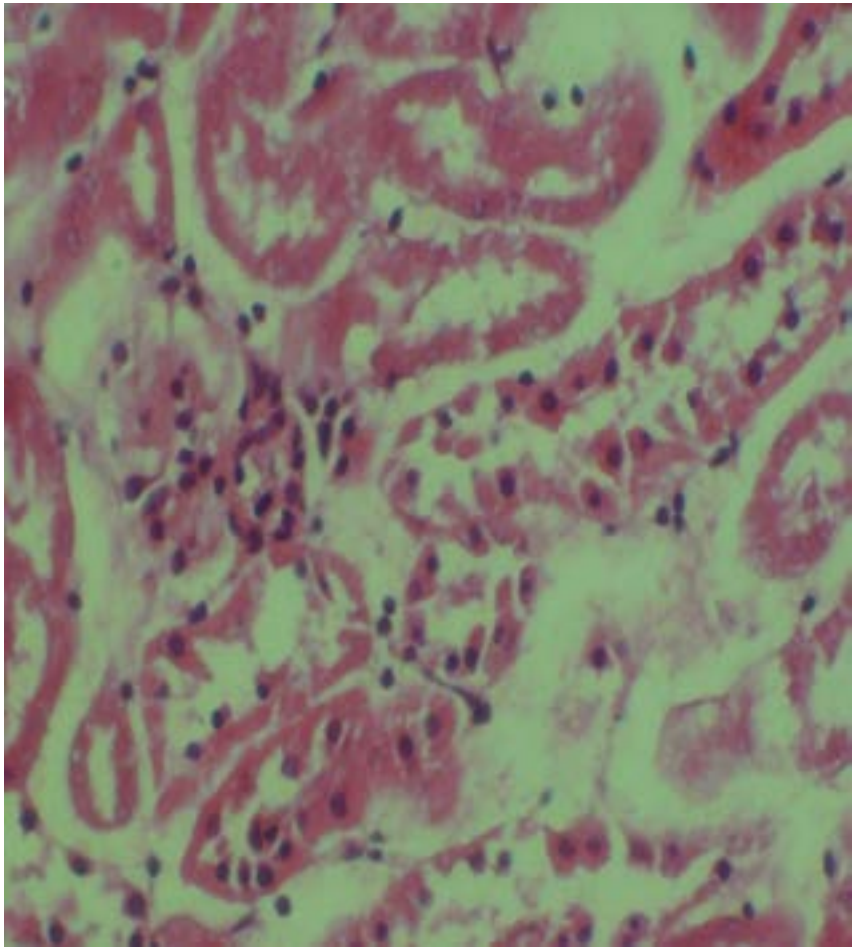
Cyanobacterial toxin concentrations in the crude extracts (three blooms)

Sample	Type of bloom	CYN Micro g/l	Deoxy CYN Microg/l	Microcystin Microg/l
1	Microcystis bloom	nil	2.1	65
2	Mixed bloom with predominantly Cylindrospermopsis	0.7	29.5	Nil
3	Lyngbia bloom	1.7	0.5	Nil

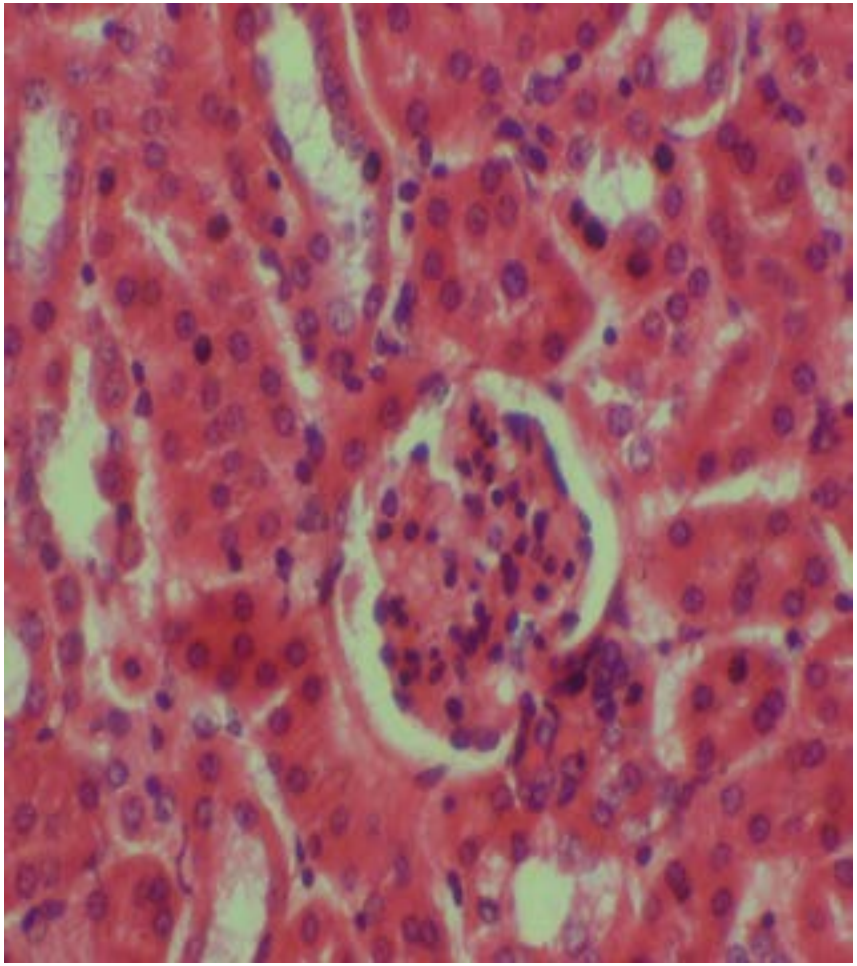
Short term effects of cyanobacterial toxin on mice kidneys

Mice group	Experiment and concentrations micro grams/L	Histology	Result
Test 1a	Diluted toxin (microcystis bloom) fed for 1 week (**Microcystin 2.28+ Deoxy CYN 0.1**)	Tubular Necrosis	**5/5**
Test 1b	Diluted toxin (microcystis bloom) fed for 1 week & water fed for 2 weeks (**Microcystin 2.28+ Deoxy CYN 0.1**)	Tubular necrosis	**2/5**
Test 2	Diluted toxin (mixed growth with cylindrospermposis) fed for 1 week (**Deoxy CYN 1.25 + Cyn 0.03)**	Tubular necrosis	**1/7**
Test 3	Diluted toxin of Lyngbia bloom fed for 1 week(**Deoxy CYN 0.024 + Cyn 0.075)**	Tubular necrosis	6/10
Control	**Water fed for 1 week**	**Normal tubules**	**10/10**

Tubular necrosis



Normal tubules in control group



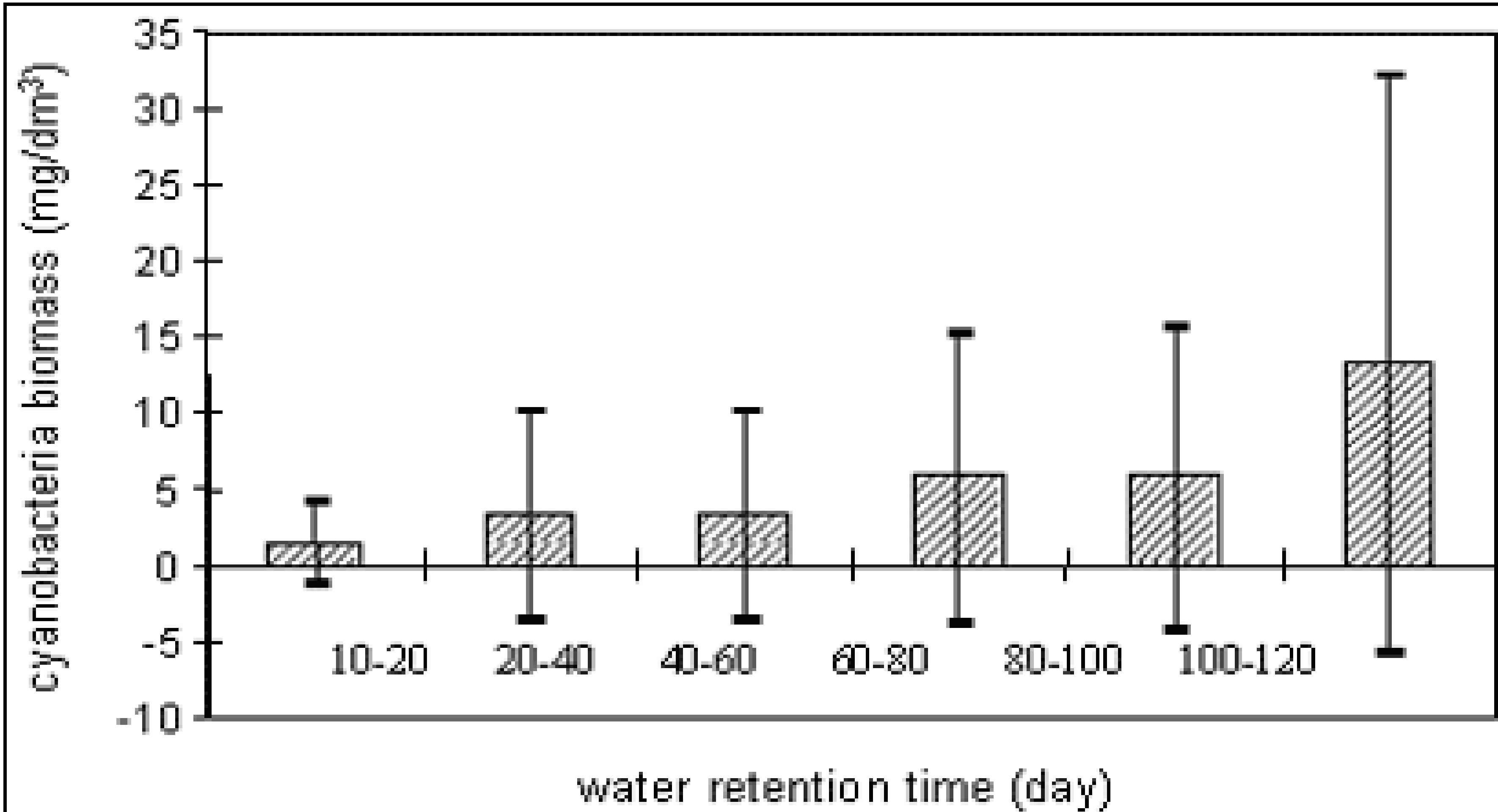
This study shows

- The ability of Cyanobacterial toxin causes acute tubular necrosis of mice.
- Two weeks after the removal of the toxin from the drinking water, there was some regeneration of the necrosed tubules.
- If the same mechanism operates in humans, we can prevent further damage / the damage could be repaired by preventing exposure to further toxin.

Why in Sri Lanka ?

- Ancient hydraulic civilization of Sri Lanka.
- Dry zone of the country has over 22000 man made water reservoirs out of which 18000 are small village tanks.
- No where else in the world we see this number of man made water reservoirs and most of them are built one millennium ago.
- The retention time of water in these reservoirs is over 9 months making it a good place for cyanobacterial growth, blooming, toxin production and concentration due to prolong dry warm weather .

Effect of longer retention time on cyanobacterial growth

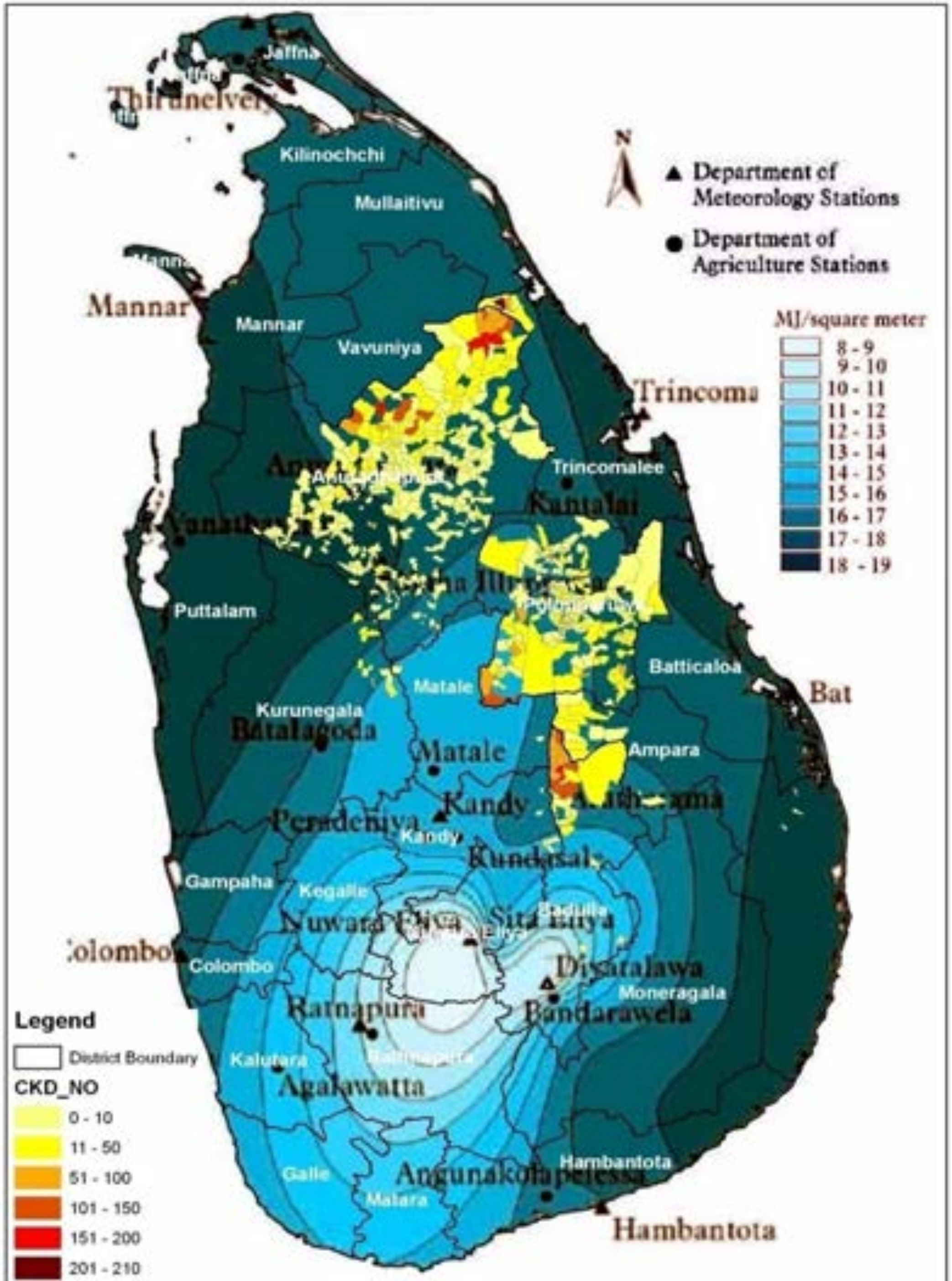


Tarczynska, M.et al, M. 2001. Proceedings of the 9th International Conference on the Conservation and Management of Lakes, ILEC, Shiga.

Why is North Central Region affected ?

- The highest number of water reservoirs are in the North Central Region.

- The studies on solar radiation of Sri Lanka shows the affected region has the highest solar radiation for several months of the year



Solar Radiation in April & Distribution of CKD-U

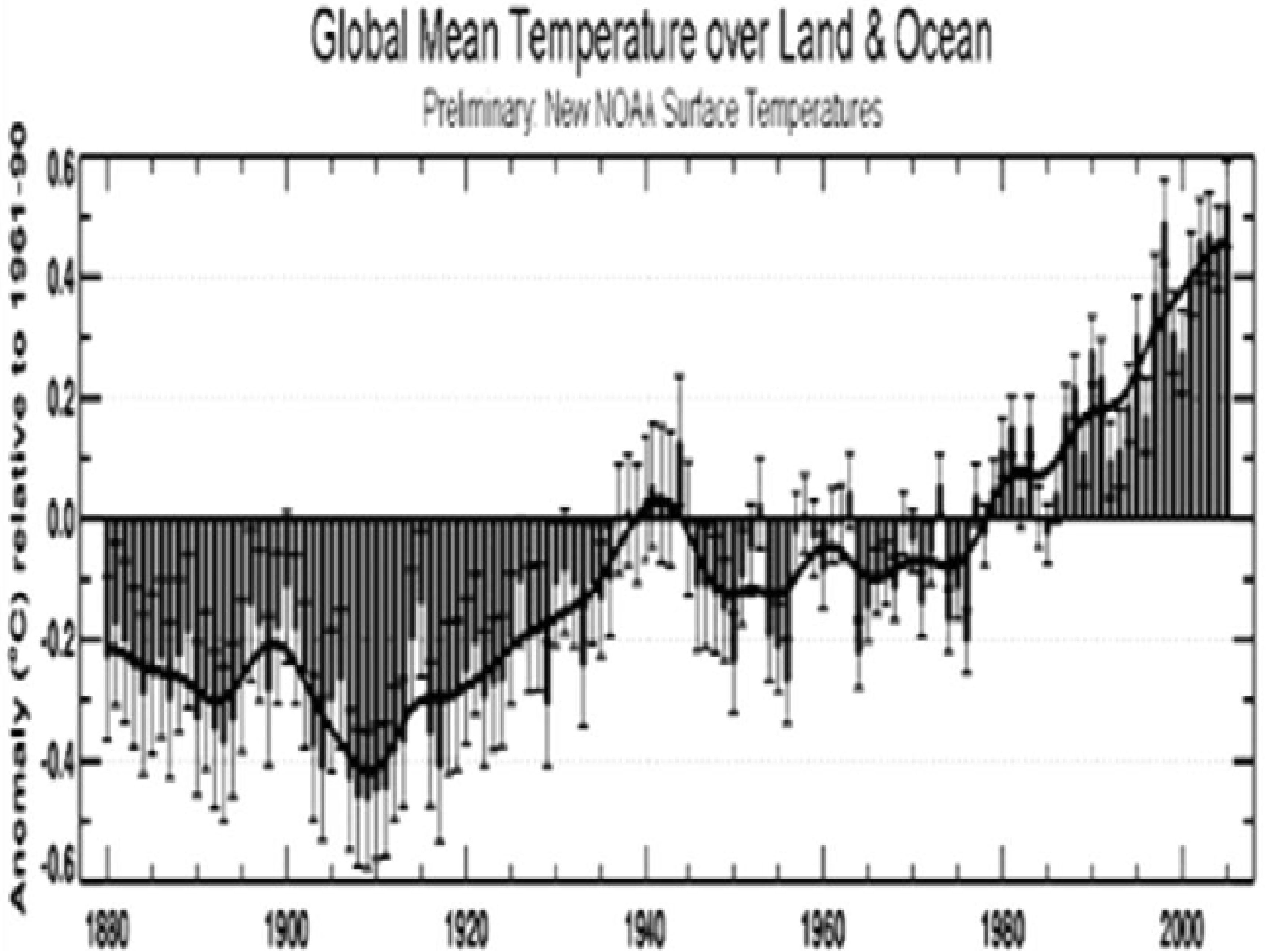
Why only some reservoirs of the NCR is affected?

- Most of the affected reservoirs
  - have a longer retention time for water than the unaffected reservoirs.
  - shallow reservoirs
- Reservoirs fed with Mahaweli water are less affected.
- The only exception is the Ulhitiya reservoir where the stagnation is likely despite the water from Mahaweli river

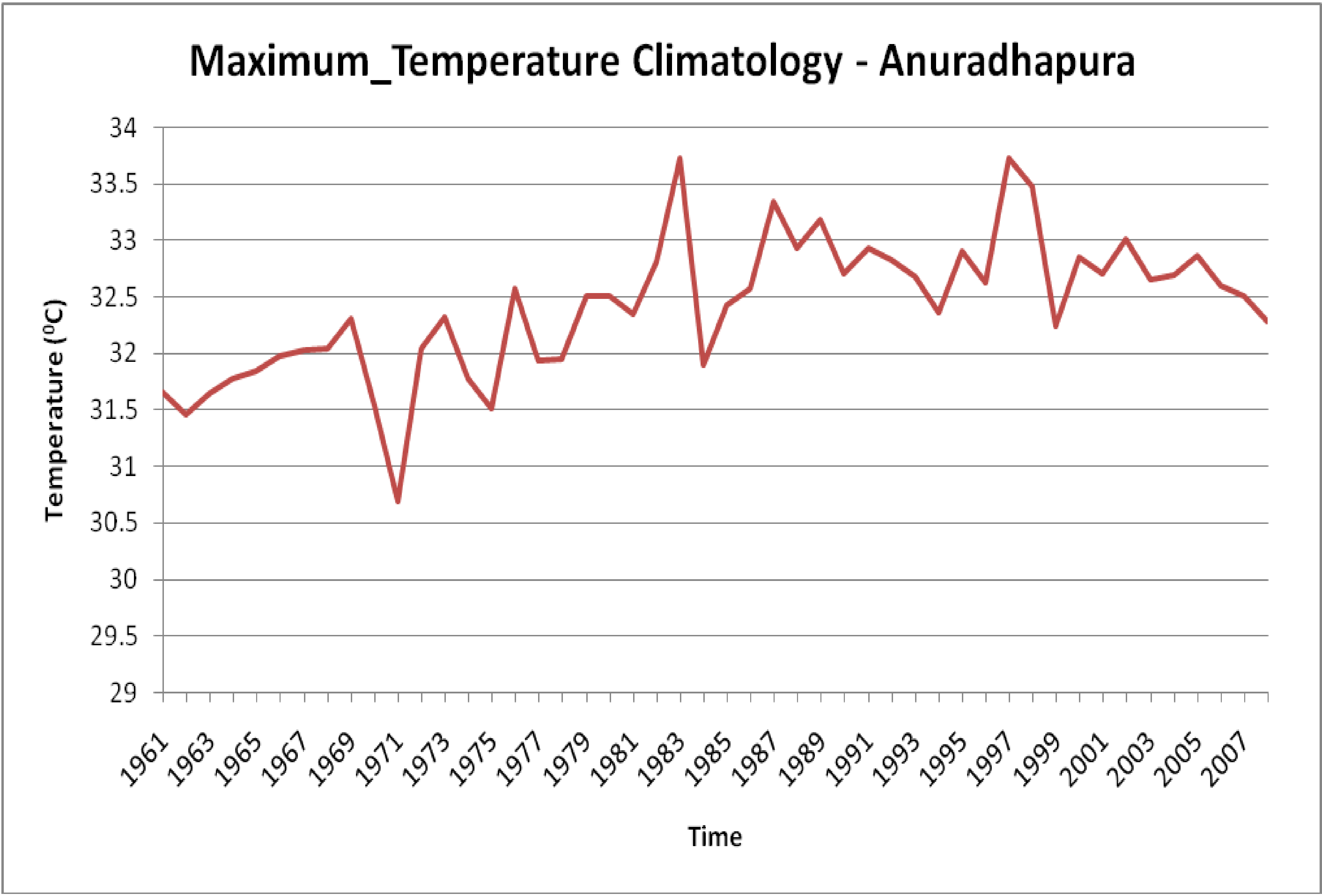
Why did CKD-U appear in early nineties?

- With global warming the temperature of the environment has increased by 0.5 degrees making cyanobacterial blooming and toxin production more likely.

- With the use of **chemical fertilizers** the **agricultural run off water contains more N P K** making reservoirs more favourable for cyanobacteria blooming and production of toxin.

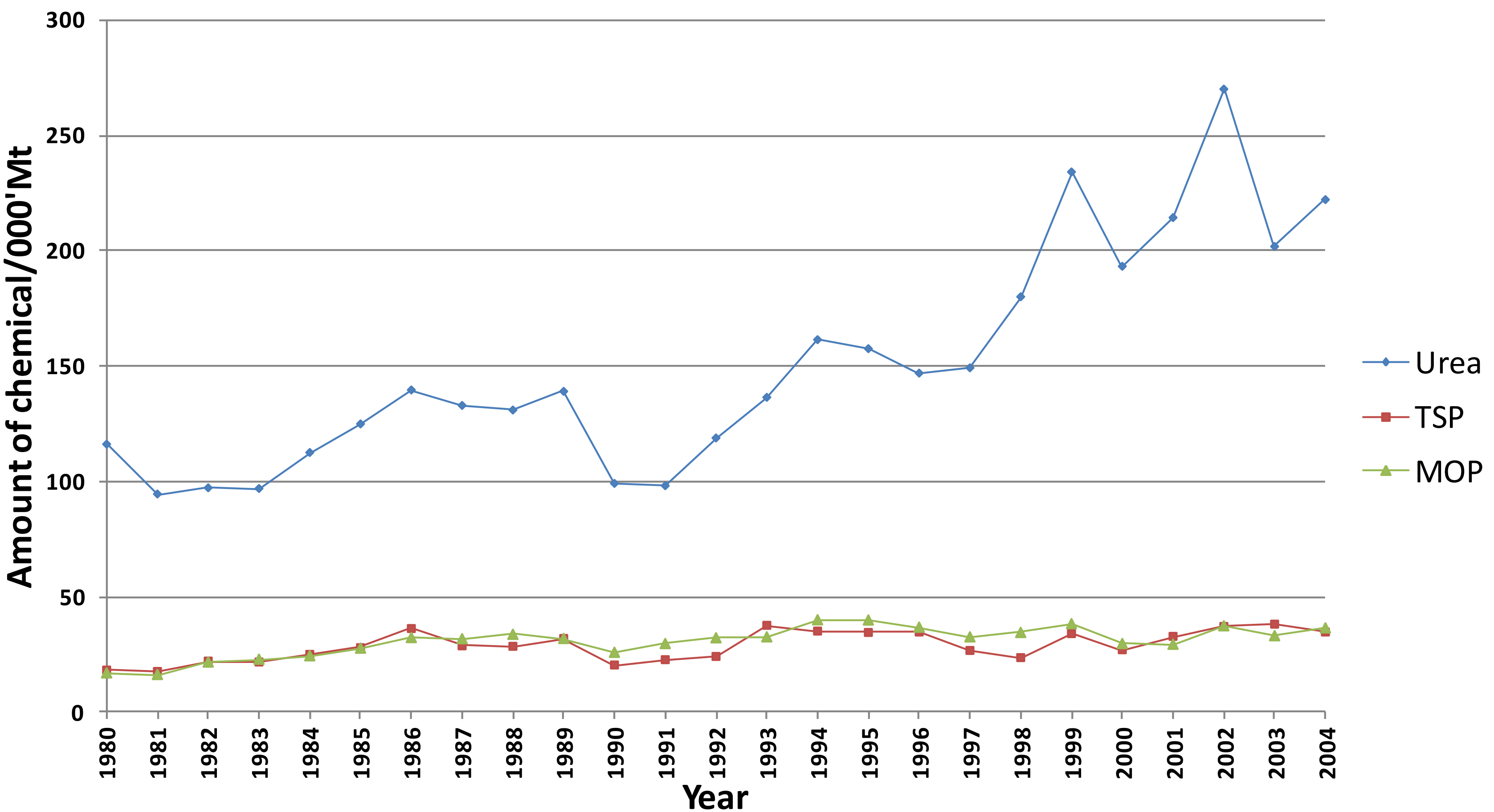


Global Mean Temperature over Land & Ocean
Preliminary: New NOAA Surface Temperatures
Anomaly (°C) relative to 1961-90
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
1880
1900
1920
1940
1960
1980
2000


Maximum_Temperature Climatology - Anuradhapura
34
33.5
33
32.5
32
31.5
31
30.5
30
29.5
29
Temperature (°C)
1961
1963
1965
1967
1969
1971
1973
1975
1977
1979
1981
1983
1985
1987
1989
1991
1993
1995
1997
1999
2001
2003
2005
2007
Time

Source: FECT

Use of chemical fertilizers in paddy cultivation 1980-2004



Source: National fertilizer registry

Distribution of chronic kidney disease of unknown aetiology