and incubated for 1 h at 37°C. The excess antibody was removed by washing twice with PBS. The slides were exposed to FITC conjugated goat anti-mouse IgG (1:100) for 1 h, which acted as the secondary antibody, washed twice with PBS and wet mounted. The slides were observed under an epifluorescence illumination (*Laborlux S, Wild Leitz, GmbH, Germany) using* Ploemopak I 2 filter block (excitation filter BP 450–490 and suppression filter LP 515). The images were recorded on Kodak gold 100 ISO films.

Epididymal spermatozoa (88–94%) from all regions showed antigen localization only in the acrosome (Figure 1); but, a small percentage of spermatozoa (4–6%) did not show fluorescence. Further, 10% of caput and 4% each of corpus and cauda epididymal spermatozoa showed fluorescence only at the tip of the acrosome. Ejaculated spermatozoa (97%) showed antigen localization in the acrosome while 3% showed localization only at the acrosomal tip.

In the present study, the immunolocalization of SP 10 in the acrosome of majority of monkey ejaculated spermatozoa is similar to that in the human[1]. During epididymal transit, the localization of SP 10 antigen in rhesus monkey spermatozoa did not show any change, indicating that this is essentially a testis-specific antigen[1] which does not undergo changes during epididymal maturation. Similar results were observed by Western blot analysis of extracts of human caput and cauda epididymal spermatozoa, using MHS 10 antibody[5]. Further, Western blot analysis of the extract of spermatozoa from baboon (*Papio papio*), cynomolgus monkey (*Macaca fasicularis*) and the human, using MHS 10 antibody, showed fourteen distinct immunoreactive bands ranging in molecular weight from 18 to 34 kD, indicating the heterogeneity in the nature of antigens in the human and the non-human primate species[6]. This antigen shows minimal or absence of cross-reactivity with somatic cells. On the basis of this observation, Anderson *et al.*[7] designated SP 10 as a primary vaccine candidate for immunocontraception. The presence of this antigen, common to spermatozoa of the human and the rhesus monkey, indicates that this is a conserved antigen. This study also shows that the rhesus monkey can be used as non-human primate model to screen the vaccine developed against SP 10 antigen, since this antigen is conserved in the rhesus monkey.

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Radium-228 in the Kaveri river ecosystem

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In this article we present the results of a study aimed at investigating the radium-228 level in water, sediment and biota (plankton, weed, bivalve, prawn and fish) of the Kaveri river ecosystem extending to a stretch of 95 km. The results show a dissolved Ra-228 concentration in river water ranging from 4.43 to 7.67 mBq/l (mean: 6.1 mBq/l). The sediment samples recorded a Ra-228 activity of 15.1 Bq/kg. The aquatic organisms demonstrated differential accumulation of the radionuclide with enhanced bioaccumulation in shells and bones. The bivalve mollusc, *Lamellidens marginalis*, was identified to accumulate higher concentrations of Ra-228 in their soft tissues (0.92 Bq/kg) and shell (3.86 Bq/kg), suggesting that they could serve as a biomonitor of Ra-228 radionuclide in a riverine system. The concentration factors (CFs) calculated for the aquatic organisms ranged from ~10 to ~10^2. However, CFs observed in shells and bones were higher than in soft tissues and muscle. Gamma spectrometry of the primordial radionuclides indicated an elevated Th-232 activity (45 Bq/kg) than U-238 activity (15 Bq/kg) in Kaveri river sediment. The significance of the results of Ra-228 radionuclide in the environment of the Kaveri river is discussed.

AQUATIC organisms display considerable ability to accumulate toxic elements and radionuclides from water, although the concentration levels of the individual element or radionuclide in the water may be exceedingly small. Reviews on the bioaccumulation by organisms of

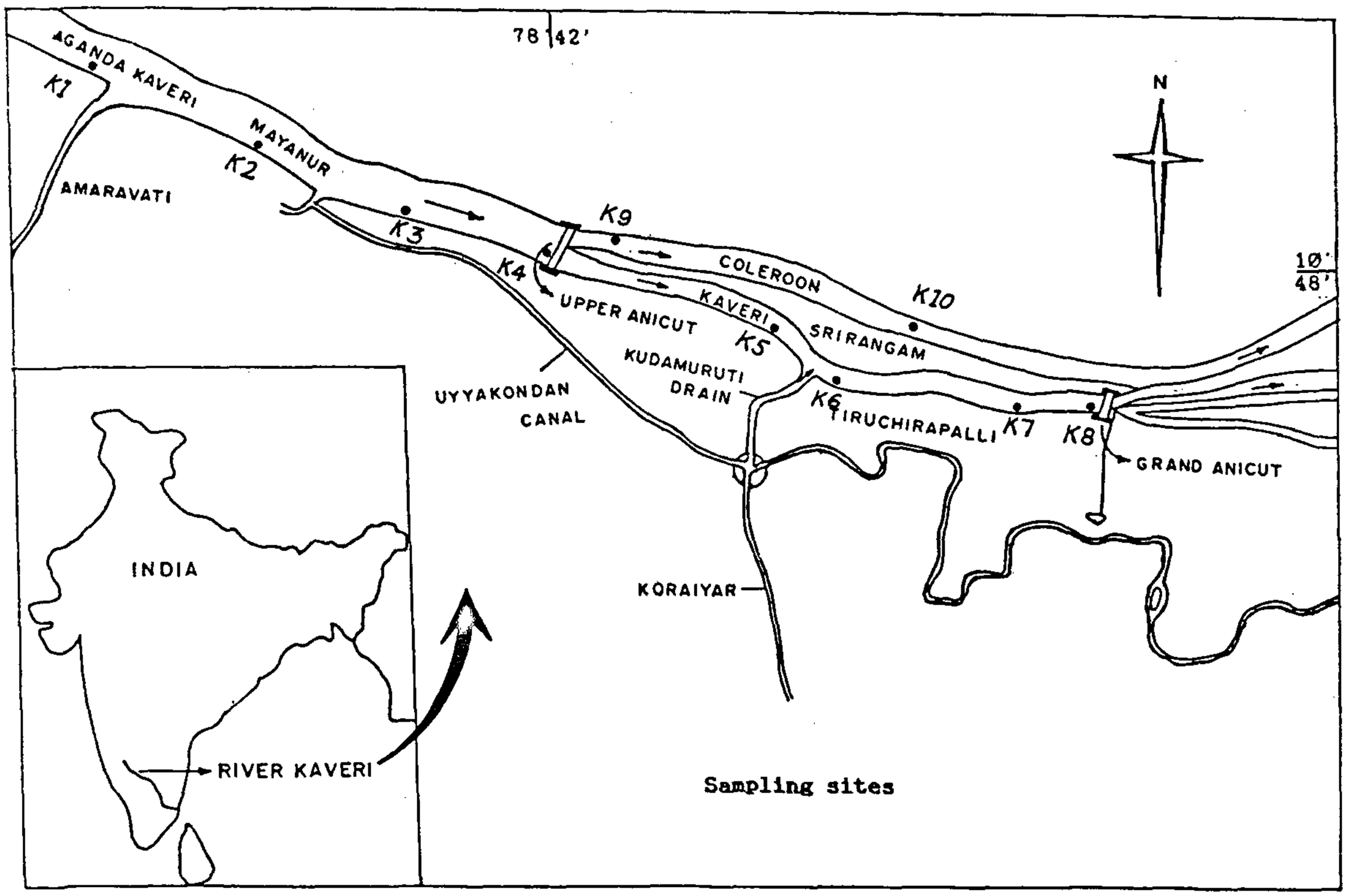

Figure 1. Location of the Kaveri river and sampling sites.

a number of trace elements and radionuclides in marine environment are available[1–4]. In contrast, information on the uptake and distribution of natural radioactivity in freshwater systems is limited[5,6]. Studies on the natural radioactivity in a freshwater system offer considerable scope for understanding the mechanism of radioactivity transfer to man through flora and fauna.

Radium-228 (half life = 5.75 yr), as a member of the primordial thorium-232 (1.4×10^{10} yr) decay chain of elements, is an ubiquitous component of the natural radiation environment, and is thus found in most abiotic and biotic components leading to direct or indirect human radiation exposure. A wide range of concentration of this isotope is encountered in the environment as a result of the varying abundance of the parent elements, differing states of equilibrium within the decay series and the nature and extent of weathering and environmental reconcentration process[5]. Uptake of Ra-228 in biological systems clearly depends upon the availability of the nuclide. Data on levels of Ra-228 in freshwater systems are scanty in the literature. In the light of the possible radioecological significance in the environment, the present study was undertaken to investigate the distribution of Ra-228 in abiotic and biotic components of the Kaveri river ecosystem, since no such data are available for this river stretch.

The area under investigation was the river Kaveri, the longest perennial river in the state of Tamil Nadu. For the present study, ten sampling stations (K1–K10) were fixed along the stretch of the river from Karur to Grand Anicut (95 km) (Figure 1). Samples of water, sediment, plankton, weed (*Eichhornia crasapies*), bivalve (*Lamellidens marginalis*), prawn (*Macrobrachium malcolmsonii*) and fish (*Mystus vittatus*) were collected at periodic intervals for two years, from August 1993 to July 1995, and analysed for Ra-228 concentrations based on the method of Iyengar *et al.*[4].

The river water (100 l) was filtered through Whatman 42 filter paper and passed through manganese impregnated acrylic fibre (25 g) packed into a glass column (75 cm long and 25 cm diameter), with a compactness just adequate to permit rapid passage of water through the column at a flow rate of 50–60 ml/min. During flow through the column, the radium isotopes were efficiently taken up and retained by Mn–acrylic fibre[5,7]. The radium-rich fibre was then washed with distilled water,

and immersed in hot 8N HCl to leach out the manganese from the fibre together with the sorbed radium activity. The lechate was evaporated to dryness on a hot plate, followed by dissolution of the residue in 2N HNO_3 and made up to a known volume (50 ml). This solution was then used for estimation of Ra-228 via Ac-228 (ref. 4).

The sediment and the biological samples were wet-weighed, dried, homogenized and transferred to a 400 ml beaker and digested with conc. HNO_3:H_2O_2 oxidizing mixture on a hot plate. After 5–6 repeat digestions, the sample was subjected to dryness, followed by successive leaching with conc. HNO_3. The residue was leached with 2N HNO_3, filtered and made up to a known volume (50 ml). The solution was then analysed for Ra-228 activity via Ac-228.

To the sample solution (50 ml), Ba (5 mg) and Pb (100 mg) carriers were added and Ra-228 was co-precipitated with $Ba(Pb)SO_4$. The sulphate precipitate carrying Ra-228 was dissolved in ammonical EDTA and $BaSO_4$ was reprecipitated with acetic acid at pH 4–5 and measured via Ac-228 (refs 4, 8, 9). Spiked experimental recoveries on water and biological samples using the above method yielded an overall efficiency of 90%. The Ra-228 concentration in all the samples has been decay corrected to the date of collection.

The concentration of primordial radionuclides in the river sediments was determined at five representative sampling stations (K1, K2, K4, K6 and K8) employing a high resolution Hyper Pure Germanium (HPGe) gamma ray spectrometer.

Table 1 shows the results of the analyses of dissolved Ra-228 activity in the Kaveri river water. The Ra-228 concentration in river water was in the range 4.43–7.67 mBq/l (mean: 6.1 mBq/l). The dissolved radionuclide concentrations in river water are higher than those reported for the Ganges (1.63–2.6 mBq/l)[10] and Amazon river (0.53–2.17 mBq/l)[11] and compare well with the values of the Sabarmati river (5–10 mBq/l)[10]. However, the levels are less than those reported for the Periyar river of Kerala (14.8–133.2 mBq/l)[12]. Moore[13] has reported

Table 1. Ra-228 activity in the Kaveri river water

Sampling station	Ra-228 activity (mBq/l)
K1	4.43 ± 0.9
K2	5.79 ± 1.4
K3	5.32 ± 1.2
K4	7.67 ± 1.4
K5	6.68 ± 1.7
K6	6.27 ± 1.6
K7	5.27 ± 1.7
K8	7.32 ± 1.2
K9	5.98 ± 1.1
K10	6.20 ± 1.9
Range	4.43 – 7.67
Mean ± SD	6.1 ± 1.0

Table 2. Activities of U, Th and Ra-228 in the Kaveri river sediments

Sampling station	Activity (Bq/kg dry)			Ra-228/Th-232 activity ratio
	U-238	Th-232	Ra-228	
K1	16.1 ± 1.1	49.3 ± 1.3	10.3 ± 2.6	0.21 ± 0.05
K2	12.4 ± 1.0	30.4 ± 0.9	12.1 ± 2.1	0.40 ± 0.07
K3	NM	NM	13.8 ± 2.4	–
K4	21.2 ± 1.4	52.3 ± 1.3	20.2 ± 3.1	0.39 ± 0.06
K5	NM	NM	21.1 ± 3.9	–
K6	10.5 ± 1.2	33.8 ± 1.1	12.7 ± 2.4	0.38 ± 0.07
K7	NM	NM	10.8 ± 2.8	–
K8	17.1 ± 1.4	59.4 ± 1.5	22.3 ± 4.6	0.38 ± 0.08
K9	NM	NM	12.1 ± 2.9	–
K10	NM	NM	15.1 ± 3.4	–
Mean ± SD	15.5 ± 4.2	45.0 ± 12.4	15.1 ± 4.5	

NM = Not measured.

that the longer a volume of water remains in contact with Th-232-bearing sediments, the more is the Ra-228 accumulated in water. Table 2 shows the results of the concentration of primordial radionuclides in the sediment samples of the Kaveri river determined by gamma spectrometry. It can be seen from the table that the activity of Th-232 is relatively higher, compared to that of U-238. Th-232 activity in the Kaveri river sediment ranged from 30.42 to 59.38 Bq/kg (mean: 45.0 Bq/kg) while U-238 activity ranged from 10.48 to 21.16 Ba/kg (mean: 15.5 Bq/kg). Hence it is suggested that the higher thorium activity in river sediments may be responsible for the observed higher concentration of Ra-228 in river water as shown by the present study. The Ra-228/Th-232 (activity ratio) range from 0.2 to 0.4, which suggests that the Ra-228 diffuses out of the sediments, and that is the source of Ra in the water.

Table 2 shows the results of Ra-228 analysis in the sediment samples of the Kaveri river. The Ra-228 activity in the river sediments ranged from 10.31 to 22.32 Bq/kg with a mean value of 15.1 Bq/kg. The level of Ra-228 in the sediment samples of the present study is in broad agreement with the findings of the similar study by Paul and Pillai[5] in the Periyar river of Kerala. In a natural environment, rocks undergo a continuous process of weathering, which eventually results in soil formation. During the weathering and migration of radium from rock to soil, radium is expected to move out in the particulate phase[14]. However, that part of the radium which tends to be solubilized in water, moves along with the water stream until it is finally deposited in the soil through chemical or biological action. The enhanced nuclide activity observed in the river sediments of the present study can be explained on the basis of high nuclide availability and the preferential uptake of Ra-228 by the river sediments. The average concentration factor (K_d factor) recorded for Ra-228 in sediment sample is 2.48×10^3.

Table 3. Ra-228 activity in the Kaveri river biota

Sample	Ra-228 activity (Bq/kg fresh)	Concentration factor*
Plankton	4.12 ± 1.6	6.75×10^2
Weed		
(*E. crasapies*)		
Root	5.15 ± 2.4	8.44×10^2
Shoot	0.58 ± 0.2	9.51×10^1
Bivalve		
(*L. marginalis*)		
Soft tissues	0.92 ± 0.3	1.51×10^2
Shell	3.86 ± 0.8	6.33×10^2
Prawn		
(*M. malcolmsonii*)		
Muscle	0.89 ± 0.4	1.43×10^2
Exoskeleton	2.97 ± 0.9	4.88×10^2
Fish		
(*M. vittatus*)		
Muscle	0.48 ± 0.1	7.90×10^1
Bone	3.77 ± 1.0	6.18×10^2

No. of samples = 10 each.

$$*CF = \frac{\text{Ra-228 activity in biota (Bq/kg fresh)}}{\text{Ra-228 activity in water (Bq/l)}}.$$

Table 3 presents the results of the bioaccumulation of Ra-228 in the biotic components of the Kaveri river. It shows varying degrees of concentration of the radionuclide within the living systems. Three observations emerge from Table 3.

a. The lower trophic organisms, such as plankton and macrophytes show relatively stronger Ra-228 accumulation trends compared with higher organisms such as mollusca, crustacea and fish.
b. Among higher animals, the general pattern was that the hard parts such as shells and bones accumulate relatively more Ra-228 as against low levels of radium observed in the soft tissues.
c. The concentration factor of Ra-228 for the riverine biota is in the range ~10 to ~10^2.

In the present study, the plankton samples registered a Ra-228 activity of 4.12 Bq/kg and a concentration factor of 6.75×10^2. The root of the aquatic weed *E. crasapies* showed a higher (5.15 Bq/kg) concentration of Ra-228 than its shoot (0.58 Bq/kg). The reported values of Ra-228 concentration in plankton samples of the present study are higher than those observed for the plankton samples of the Hudson river (2.26 Bq/kg)[15] and far less than the activity recorded for the plankton samples of Kalpakkam coastal waters (18.5 Bq/kg)[16]. However, the activity of Ra-228 observed in the aquatic weed agrees well with the data of other investigators[16–18]. In plankton and macrophytes, direct adsorption of radioelements from water to the outer surface is far more likely to occur, in view of their high surface-to-volume ratio. Among aquatic fauna, relatively higher radioactivity accumulations are generally observed in molluscs as compared with fish. Within animals, bivalve mollusc showed a greater (soft tissues: 0.92 Bq/kg; shell: 3.86 Bq/kg) accumulative capability than prawns (muscle: 0.89 Bq/kg; exoskeleton: 2.97 Bq/kg) and fish (muscle: 0.48 Bq/kg; bone: 3.77 Bq/kg). It is a well-known fact that muscles are used as indicators[19] of heavy metals and radionuclides in the environment. The reason being that suspension-feeding bivalve molluscs ingest detritus materials with a high degree of radionuclide accumulation, and are capable of accumulating contaminants in their body organs to a concentration significantly higher than in the ambient water, thereby facilitating analysis of impact of these contaminants in their body organs to a concentration significantly higher in biological systems[19,20]. Enrichment of radium in shells and bones of aquatic organisms has already been reported[14] and it is also evident from the present study. During metabolism, radium is expected to take the calcium pathway and accumulate in calcium-rich tissue. Maybe Ra co-precipitates when the bone grows[14]. However, there appears to be a considerable variability in the enrichment of radium relative to calcium among different biological species tested. In the case of mollusca, crustacea and fish, the radium present in water may enter the animal by one or more routes. In general, three distinct environmental processes, viz. adsorption, absorption and ingestion, have been mentioned by most investigators to be responsible for the entry of radioactive elements in organisms. These mechanisms operate to various degrees both individually and collectively, since an organism is capable of accumulating an element from the aquatic environment by either single or multiple routes. Further, the elemental accumulation in organisms can take place as a result of passive or active uptake process. The major route of radium entry in organisms, like any other nuclide is by ingestion of food. In addition, direct absorption through the surface of gills, the mouth or other external epithelia also facilitates entry of the radionuclide. The muscle of fish generally displayed very poor radium concentration levels with low concentration factors (CFs). The hard parts such as shell and bone, which are calcareous in nature, are characterized by relatively higher CFs in comparison with soft tissues of animals tested.

The results of the present study provide a database on the activity concentration of natural β-emitting radionuclide, i.e. Ra-228 in the Kaveri river ecosystem. In the absence of any man-made radioactivity input, the Ra-228 activity in river water shows a higher order of activity which depends upon the source characteristics in the study area. Ra-228 activity in river water was higher in summer months during lean flow since it involves subsurface waters, which in general have higher Ra isotopes. The aquatic organisms show an appreciable potential for accumulating radium from the environment. The bivalve mollusc, *L. marginalis*, exhibit good

accumulation of Ra-228 (CF: 151–633), suggesting that they could serve as a useful indicator species for radionuclides like Ra-228 in any riverine system. Since the river food organism accumulate Ra-228 in the edible part, namely muscle, at a relatively low level, the dose transfer to man by ingestion route is negligible.

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Modulating effect of gelonin gp330 conjugate on Heymann's nephritis induced in rats – A pathomorphological evaluation

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Heymann's nephritis (HN) in rats induced by injecting renal proximal tubule brush border protein gp330, is an animal model replicating human autoimmune membranous glomerulonephritis[1]. Endogenous IgG gets deposited between the foot processes in the epithelial side of the glomerulus and causes complement-mediated membrane injury, leading to proteinuria and basement membrane thickening. We investigated the effect of a toxin, gelonin conjugated to gp330 and targetted against antigp330-producing cells in ameliorating immune injury and nephrotic state in rats. The groups of animals injected with purified gp330 revealed by immunofluorescence, characteristic granular deposits of IgG along the basement membrane. The rats intravenously injected with gelonin gp330 conjugate, four days after the antigenic challenge with gp330 in two doses, showed amelioration of the nephrotic state and appreciable reduction in glomerular IgG deposits against immune injury. This substantiates our earlier biochemical results and corroborates the possibility of using toxins conjugated to specific antigen in treating antibody-mediated autoimmune diseases.

IDIOPATHIC diffuse membranous glomerulonephritis is a chronic progressive disease. It is most common in adult men and usually presents with the nephrotic syndrome. The pathogenesis of this renal disorder is not known. In animals, lesions closely resembling human membranous glomerulonephritis referred to as Heymann's nephritis (HN) can be induced by immunizing against a glycoprotein antigen, gp330, present in the brush border of renal cortical proximal tubular cells. The circulating divalent autoantibodies to gp330 cross the glomerular capillary basement membrane and get deposited between the foot processes. This antibody along with complement damage the plasma membrane of the foot processes, leading to proteinuria and diffuse membrane thickening[2]. There is a strong suspicion that an autoimmune mechanism similar to that operating in Heymann's membranous glomerulonephritis in the rat is probably responsible for

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