

## Total dissolvable copper and mercury concentrations in innershelf waters, off Kalpakkam, Bay of Bengal

Coastal areas are ideal locations for many industries, particularly power plants, both nuclear and conventional (coal/oil fired), due to abundant availability of sea water for condenser cooling. Thermal and chemical pollution are two important ecological and environmental problems associated with the use of sea water as a cooling fluid<sup>1</sup>. In a tropical environment, as in the Indian subcontinent, heating of the sea water raising its temperature a few degrees Celsius above ambient brings it close to the lethal limits for temperature tolerance for many coastal marine organisms<sup>2</sup>. The problem is sensitively compounded if the effluents contain highly toxic substances like mercury, which may endanger the endemic population of marine life. Hence, the environmental impact of waste discharged from the power plant has become an area of recent scientific interest. Therefore, the study of radioecological consequences of the nuclear industry in India has been given prime

importance and various units have been established to study environmental interactions of controlled low-level waste releases<sup>3</sup>.

The Madras Atomic Power Station (MAPS) consists of 2 reactors of 235 MW(e) each at Kalpakkam (12°33'30"N lat. and 80°11'E long.), a coastal town situated 65 km south of the city of Chennai, along the east coast of India (Figure 1). Sea water (about 35 cubic m/s) is used as the secondary coolant for the condensers as well as other auxiliary cooling systems in these reactors. The sea water intake structure for the reactor is located 420 m offshore. A 405 m long approach jetty connects the intake system to the shore. The jetty also supports a discharge pipeline for low level radioactive effluents. The warm water discharged from the reactors has a  $\Delta T$  of about 7°–10°C (ref. 4). To understand the effects of plant operations on the marine ecosystem, total dissolvable copper and mercury concentrations in

waters of the innershelf region (up to a water depth of 52 m), off Kalpakkam were studied and have been reported here.

Forty-two surface (below 1 m from sea surface) and bottom (above 1 m from sea bottom) water samples were collected using Nansen water sampler (1 litre capacity) from 42 stations along 6 traverses, during pre-monsoon (September 1995) and post-monsoon (April 1996). Samples for metal analysis were acidified immediately using 2 ml of conc. HNO<sub>3</sub> per litre of sample to prevent sample deterioration and kept in a refrigerator until analysis. The liquid-liquid extraction procedure<sup>5</sup> was used to determine the metals in sea water. Trace metals from 100 ml of sea water were preconcentrated by complexing with ammonium pyrrolidinedithiocarbamate (APDC), extracted with iso-butyl methyl ketone (IBMK), and sequentially back extracted by 14 M HNO<sub>3</sub>, 12 M HCl and high-purity water (HPW). The final solutions obtained from the sam-

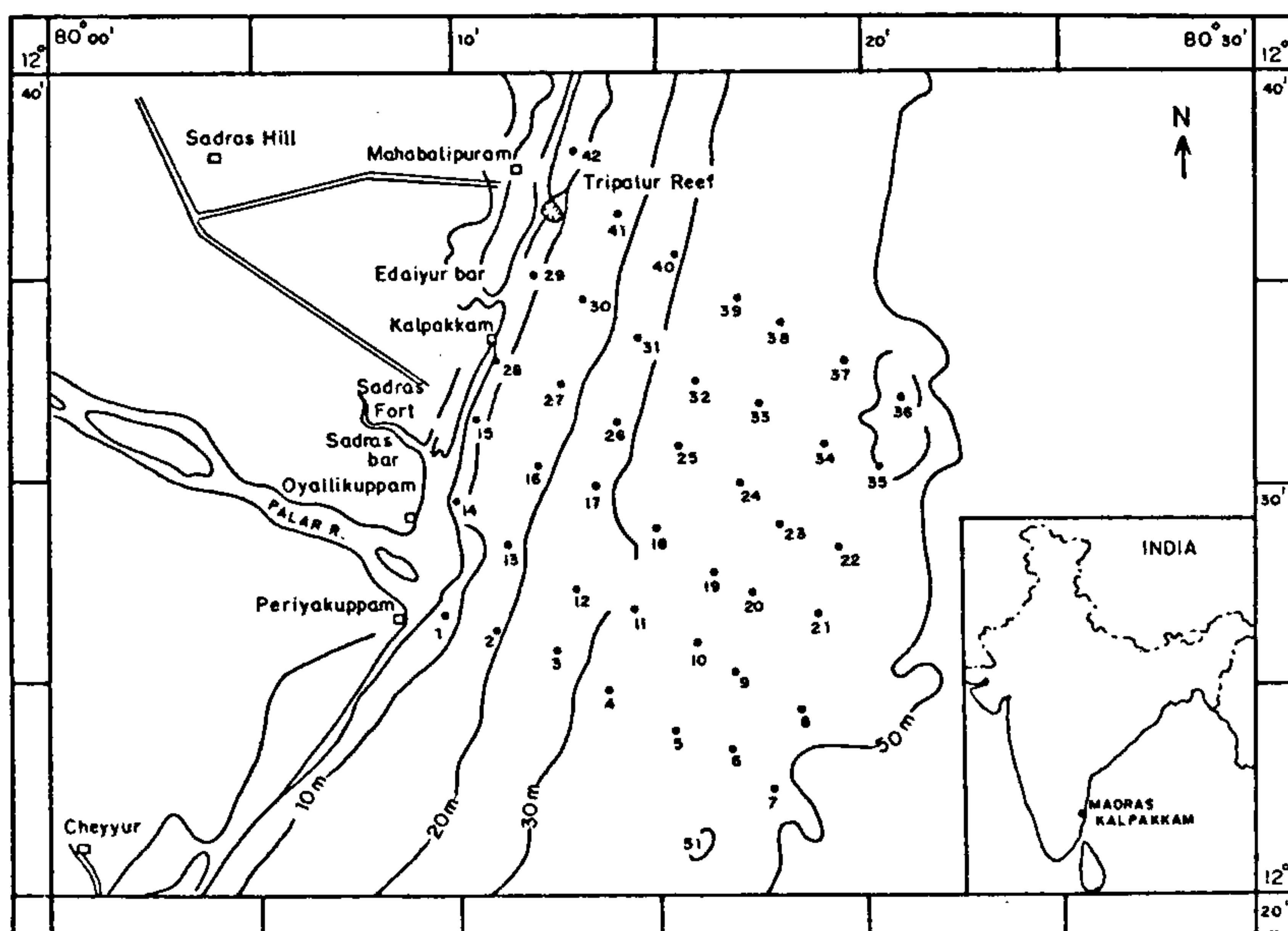


Figure 1. Study area and sampling stations.

**Table 1.** Analytical values of total dissolvable copper and mercury from innershelf waters, off Kalpakkam

Station no.	Depth (m)	Cu				Hg			
		PRM		POM		PRM		POM	
		Surface	Bottom	Surface	Bottom	Surface	Bottom	Surface	Bottom
1	7	239	127	1256	728	269	872	109	112
2	15	255	176	1784	952	432	982	-	146
3	21	236	119	2029	710	478	1112	-	-
4	28	192	78	1432	518	134	340	-	-
5	35	264	76	1261	350	327	627	-	-
6	50	245	86	884	1050	213	-	97	-
7	52	152	95	382	165	244	424	202	311
8	52	127	76	165	110	133	687	-	106
9	40	191	264	462	2844	1284	1344	-	44
10	38	2565	942	531	878	1041	404	-	-
11	34	117	1374	881	643	966	1512	-	-
12	30	747	119	434	321	994	1548	-	98
13	20	459	192	200	98	987	945	-	206
14	14	1232	240	153	129	821	1352	-	316
15	11	273	295	175	365	1408	1664	-	-
16	17	770	252	553	696	429	174	-	-
17	21	437	728	187	878	272	1000	-	-
18	28	356	171	244	594	369	858	278	314
19	34	428	263	638	308	637	1303	279	-
20	43	502	250	957	1522	1054	625	312	-
21	51	244	193	408	1122	1014	309	-	224
22	52	166	91	219	127	183	683	321	-
23	33	291	250	1325	738	1364	1046	274	304
24	27	183	163	649	413	1513	1008	-	255
25	24	214	153	270	177	1426	1011	-	308
26	18	535	210	1103	250	705	1236	-	-
27	16	158	185	204	969	450	1400	403	-
28	13	180	578	469	744	1041	1472	526	-
29	9	164	492	315	235	973	1523	517	-
30	13	162	197	85	163	1074	1611	-	-
31	17	143	123	64	115	823	1534	-	218
32	26	142	166	148	275	827	1215	-	-
33	34	171	199	240	271	615	848	104	-
34	41	127	108	539	875	544	648	126	321
35	49	93	78	541	1107	632	1039	87	112
36	49	89	85	488	1002	1304	1425	109	107
37	33	101	96	511	318	1248	1317	96	-
38	27	164	162	585	189	1416	1635	-	47
39	26	72	89	112	81	863	941	-	-
40	17	131	62	164	223	282	315	-	-
41	13	107	153	135	96	64	566	-	-
42	9	202	164	106	641	322	747	-	-
Mean		326.57	236.19	554.48	571.19	742.26	983.38	91.43	84.50

All values are expressed in  $\mu\text{g l}^{-1}$ .

PRM, Pre-monsoon (September 1995); POM, Post-monsoon (April 1996).

Depth for bottom waters only.

ples were analysed for total dissolvable copper and mercury using an ICP-AES. Recovery values as the mean for three replicates  $\pm$  relative standard deviation for Cu and Hg are  $96 \pm 3.6\%$  and  $77.4 \pm 2.2\%$ , respectively. Precision of the analysis was checked with Standard Reference Material BCSS-1 and BEST-

1 from the National Research Council of Canada.

The results of the present study (Table 1) show Cu in the range 72–2565  $\mu\text{g l}^{-1}$  and Hg in the range 64–1513  $\mu\text{g l}^{-1}$  for pre-monsoon surface waters. In bottom waters, more or less the same values were obtained for Hg in

the range 0–1664  $\mu\text{g l}^{-1}$ , but comparatively lower values were measured for Cu in the range 62–1374  $\mu\text{g l}^{-1}$ . In the post-monsoon surface waters, Cu in the range 64–2029  $\mu\text{g l}^{-1}$  and Hg in the range 0–526  $\mu\text{g l}^{-1}$  were measured. Bottom waters show higher values of Cu (81–2844  $\mu\text{g l}^{-1}$ ), whereas lower values were shown by Hg (0–321  $\mu\text{g l}^{-1}$ ). Very high values of the elements reveal anthropogenic input from the coastal industries.

The reported values of Cu in coastal waters of Goa<sup>6</sup> (0–69.77  $\mu\text{g l}^{-1}$ ), Arabian Sea<sup>7</sup> (av.  $4.7 \pm 0.4 \mu\text{g l}^{-1}$ ), upper layers of Laccadive Sea<sup>8</sup> (av.  $13.3 \pm 1.1 \mu\text{g l}^{-1}$ ) and inshore waters of western Bay of Bengal<sup>9</sup> (0.82–5.58  $\mu\text{g l}^{-1}$ ) are well below the present study values, and are higher than the range 0.2–22  $\mu\text{g l}^{-1}$  of Cu for sea water<sup>10</sup>. The very high values encountered in the analysed samples clearly reveal the point source as the power plant. Previous studies<sup>11–13</sup> have indicated that micro and macro-fouling of organisms are unavoidable problems in the study area, reducing the pressure of incoming water for cooling systems and, thereby, increasing the cost of plant operation. To control the fouling nature of organisms, antifouling paints are commonly used by many coastal industries, ships and fishing launches. The antifouling paints used for the sea water intake pipeline structure is one of the major sources of higher concentrations of copper. The high concentrations of copper leaching from the ship's antifouling paint have been reported in the Indian Ocean<sup>14</sup>.

Furthermore, surface colonization by microbial biofilms in industrial systems results in metal corrosion<sup>15</sup>. Nuclear power plants are particularly susceptible to microbiologically-induced corrosion (MIC) owing to the safety-related redundancy built into the design of many systems, which often results in low or stagnant conditions<sup>16</sup>. Due to the highly corrosive nature of sea water, copper-based alloys such as cupronickel, aluminium and admiralty brasses are generally used in heat exchangers of power plants<sup>17</sup>. Most of the organisms that colonize metal surface produce polymers, and exopolymers produced by sediment bacteria promote corrosion and, in turn, deterioration of copper alloys<sup>18</sup>. According to Little *et al.*<sup>19</sup> and Bremer and Geesey<sup>20</sup>, the release of

bacterial metabolites and biopolymers with differing electrical charges and composition is sufficient to develop corrosion potential within the biofilm on the copper. Energy dispersive X-ray analyses (EDAX) have shown that the corrosion products are oxides of copper<sup>21</sup>, and Jolley *et al.*<sup>22</sup> have observed release of copper ion due to marine fouling and corrosion of copper and its alloys. Hence, Cu in the study area is considered as high, mostly due to anti-fouling paints and corrosion of copper alloys and related copper ion release.

Mercury shows good seasonal variation between the study seasons. In the pre-monsoon, both the surface and bottom waters displayed higher values from 64 to 1513, and 0 to 1664  $\mu\text{g l}^{-1}$ , respectively, which are 3–5 times higher when compared to post-monsoon surface (0–526  $\mu\text{g l}^{-1}$ ) and bottom waters (0–321  $\mu\text{g l}^{-1}$ ), indicating that the distribution of mercury is mainly controlled by hydrography of the area. The seasonal and spatial distribution of trace metals in relation to hydrography is discussed in another paper<sup>23</sup>. The influence of hydrographic parameters on the distribution of this metal has been confirmed by the nonavailability of Hg at many stations during post-monsoon (Table 1).

Mercury concentration at the surface of the open ocean is in the range 10–100  $\text{ng l}^{-1}$  (ref. 24). Examining the data available from 1934 to 1972, Fitzgerald and Lyons<sup>25</sup> had reported mercury concentrations in sea water to range between 3 and 364  $\text{ng l}^{-1}$ . Chester *et al.*<sup>26</sup> reported mercury concentration at the surface to range from 0.5 to 127  $\text{ng l}^{-1}$  from samples of different oceans including southern Indian Ocean. In the coastal waters of the Arabian Sea, the average surface value was 136  $\text{ng l}^{-1}$ , and for the entire Arabian Sea, the average value at the surface was 120  $\text{ng l}^{-1}$  (ref. 27). Average surface concentration of Hg in the Laccadive Sea was 91  $\text{ng l}^{-1}$  (range 60–120  $\text{ng l}^{-1}$ ) and, pooling the observations of all the cruises, the average concentration at the surface for the northern Indian Ocean is 106  $\text{ng l}^{-1}$  (ref. 28). Mercury levels in sea water have been reported as negligible, 1033  $\text{ng l}^{-1}$  off the Tuticorin coast, one of the heavily industrialized areas, situated in the Gulf of Mannar<sup>29</sup>. The reported range values of other

oceans ( $\text{ng l}^{-1}$ ) include those of the Atlantic, 0.5–225 (ref. 26); Pacific, 12–153 (ref. 25) and China and Japan Seas, 6–51 (ref. 26). All the above values are well below the present results and the higher values of Hg indicate the influence of power plant operation on coastal waters.

More than 80 different industrial processes require mercury either as raw material or catalyst, of which the most important industries utilizing mercury are chlorine and alkaline plants for electrolytic production of chlorine and caustic soda and plastic industries for catalytic reactions<sup>30,31</sup>. Most of the mercury pollution is probably due to chlorine and cellulose industries<sup>32</sup>. To combat biodeterioration of cooling systems, conventional chemical methods involve dosing with toxic substances such as chlorine, bromine, chlorine dioxide, ozone, acrolin, etc.<sup>1</sup>. Development of a satisfactory control strategy requires a thorough understanding of the biological and environmental factors operating in the area. To control bio-fouling and biocorrosion, chlorine has been effectively used intermittently with antifouling paints in the study area. Therefore, electrolytic production of chlorine has been considered as the most likely source of higher values of Hg. The present values correlate well with the earlier report consisting of analytical values of intertidal sediments from south-east coast of India by Subramanian and Mohanachandran<sup>33</sup>. According to them, mercury shows the maximum value at Mahabalipuram (Mamallapuram) near Chennai (560  $\mu\text{g kg}^{-1}$ ). Sediment mercury distribution study of Sasamal *et al.*<sup>34</sup> clearly shows higher values in the east coast of India. They claimed that coastal industries, including six chlor-alkali plants with mercury cell processing systems are sources for this metal. Zingde and Desai<sup>35</sup> reported 0.17–8.21  $\mu\text{g g}^{-1}$  of mercury in the surficial sediments of Thane Creek, Mumbai, an area incorporated with power plants. Discharge of mercury-containing effluents into coastal waters of Mumbai from chlor-alkali industries leading to fish kill has also been reported<sup>36</sup>. Hence, high concentrations of total dissolvable copper and mercury suggest that the coastal waters are influenced by the coastal industry in the area under investigation.

As this is the first report, it is realized that this preliminary study needs to be expanded with other trace metals in coastal waters as well as in sediments and biota.

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## Stem disc culture: Development of a rapid mass propagation method for *Dendrobium moschatum* (Buch.-Ham.) Swartz – An endangered orchid

The clonal propagation from *Cymbidium* apical shoot meristem by Morel<sup>1</sup> has been the most sensational development which has revolutionized the orchid industry and triggered global expansion of tissue culture for rapid propagation. The technique has been especially important for orchids as their genotypes are highly heterozygous and sexual reproduction is an extremely slow process. Morel estimated that it is possible to obtain more than four million plantlets in a year from a single explant<sup>2</sup>. Among different methods available<sup>3,4</sup>, shoot tip and axillary bud cultures are widely used<sup>5</sup>. Regeneration potential of alternative plant parts other than shoot tip and axillary buds like stem, leaf, root and inflorescence have been worked out in some species of orchids<sup>6–17</sup>. Both shoot tips and axillary buds produce protocorm-like bodies (PLBs) which subsequently develop into plantlets. The clonal propagation from shoot meristem culture has proved dis-

advantageous particularly for endangered orchid taxa because continuous excision of meristematic region may threaten the existence of the mother plant. So, the emphasis has now been shifted towards faster methods by exploring alternative plant parts whereby the mother plant can be saved<sup>17</sup>. Studies on rapid regeneration have been scanty in view of the success reports in few orchid taxa when compared to the large size of the orchid family<sup>5,18</sup>.

The present investigation deals with the development of a rapid regeneration method using thin sections of stems from *in vitro* raised seedlings of *Dendrobium moschatum* (Buch.-Ham.) Swartz, an epiphytic endangered orchid taxon<sup>19</sup>. During last two decades extensive work has been done on micropropagation of orchids and vast literature has been accumulated in this area. However, information in this regard in *D. moschatum* (Figure 1a) is entirely lacking. Hence, the present

work was undertaken with a view to finding out a suitable method for rapid micropropagation of this orchid taxon.

Shoots of 6–8-week-old *in vitro* raised seedlings of *D. moschatum* were used as explant source in the present study. Shoots were taken out of conical flasks aseptically and the leaves and roots were removed. Transverse sections measuring 1–1.5 mm were prepared by cutting the stem with a sharp, sterile surgical blade. Two different basal media, Vacin and Went<sup>20</sup> and Knudson C (KnC)<sup>21</sup> both in liquid (without agar) and semisolid (with agar) form were tested. A positive and most encouraging result was obtained in KnC medium. Both the media were suitably modified. The modified media were supplemented with coconut milk (CM, 15% v/v), various concentrations of indoleacetic acid (IAA; 0.5, 1.0, 2.0 and 3.0 mg l<sup>-1</sup>), 2,4-dichlorophenoxyacetic acid (2,4-D; 0.5, 1.0, 2.0 and 3.0 mg l<sup>-1</sup>), and different combinations of a naphthaleneacetic