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ACKNOWLEDGEMENTS. We thank members of our laboratory for discussions. B.D.P. is a recipient of Senior Research Fellowship from Council of Scientific and Industrial Research, Government of India. The work is supported by a grant from Technology Development Mission, Government of India and Bangalore Genei Pvt. Ltd. to V.N.

Received 5 March 1999; revised accepted 17 May 1999

Metal toxicity and trace element deficiency in some wild animal species from north-east India, as revealed by cellular, bio-inorganic and behavioural studies

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Toxicity of some heavy metals (lead, mercury, selenium) and deficiency of some essential trace elements (chromium) have been detected in some wild animal species from north-east India with the help of inductively coupled plasma spectroscopy of hair and bone, cellular and surface ultrastructural features of skin and hair and behavioural studies on symptoms related to toxicity and deficiency of some elements. The values of elemental content indicating their toxicity or deficiency were found to be statistically significant. Electron microscopic studies on cellular and ultrastructural features of skin and hair revealed specific toxic and deficiency effects of some elements. Behavioural studies indicated several symptoms related to certain elemental disturbances, viz. loss of appetite, constipation, salivation, photophobia, tendency to wander in a circle, etc. The possible source of toxicity and deficiency of the element were examined by analysing soil and water samples from the home range of the animals and also by studying the behaviour pattern of animals in relation to mobility, migration and sequence of movements.

THE population of several wild animal species from north-east India have been reported to be declining. Although steps are being taken to protect these animals, it appears that certain important aspects such as impact of environmental pollution, physiopathologic states of animals, trace element status, etc. are missing in current conservation management strategies. Studies from this region are restricted to behaviour and ecology of only some animals with very few reports on physiology, biochemistry or pathological states[1–3]. The strong and compulsive inter-relationship among wild species, particularly the predator–prey dynamic relation, the pathological and physiological states play the most vital role in energy transfer mechanism and subsequently the survival of the species involved. Trace element nutrition and inorganic composition of the body plays a very important role in physiopathologic state and reproductive efficiency of animals. Long-time metabolic changes of various elements and present as well as past nutritional events in an individual are best reflected in the hair, which is considered as the recording filament[4,5]. Animal bones in the home range of animals also appear to be important in identifying xenobiotics introduced into the ecosystem.

The hair, skin and bone samples of the leopard cat (*Felis bengalensis*), civet cat (*Vivera zebitha*), flying squirrel (*Petaurista magnificus*) and leopard (*Panthera pardus*) were obtained from various sources. The available hair, bone and skin samples belonged to a considerable number of individual animals (ranging from 10 to 15) of different species from various locations of Assam and Meghalaya, viz. Reserve Forest near Umkiang, Jaintia hills, Meghalaya, Lailad Reserve Forest, Ribhoi, Meghalaya and Rani Reserve Forest, Assam.

The hair, bone, water and soil samples were prepared by conventional methods for elemental analysis in an inductively coupled plasma spectrometer, ICP-AES (LABTAM 8440M GBc) (Tables 1–3). The values on elemental concentration were subjected to statistical analysis by applying Student's *t*-test, and heavy metal contents of hair were compared with those of human hair in relation to toxic limit (Table 1). Cellular studies in relation to the effect of toxicity and deficiency of

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Table 1. Concentration of certain elements in unwashed and washed hair samples of wild animals, as compared to the toxicity limit of human hair. The results are mean ± SD of 10 analyses of each sample collected from different sources at different times. $P = 0.5$

Metals (µg/g)	*Felis bengalensis*		*Panthera pardus*		*Vibera zebitha*		*Petaurista magnificus*		Toxic limit for human hair (µg/g)	Ref.
	unwashed	washed	unwashed	washed	unwashed	washed	unwashed	washed		
Lead	110 ± 20	87 ± 6	ND	ND	19.5 ± 2	14.3 ± 2	45 ± 5	42 ± 5	34 ± 0.5	21
Chromium	78.4 ± 5	72.3 ± 7	ND	ND	ND	ND	ND	ND	81 ± 2	21
Mercury	168 ± 10	161 ± 15	93.6 ± 2	91.8 ± 3	128 ± 10	125 ± 15	74.4 ± 2	72.0 ± 5	50–125	7
Selenium	82 ± 10	73 ± 10	53 ± 10	51 ± 8	50 ± 5	45 ± 7	84 ± 3	83 ± 5	32.2	7

Table 2. Elemental concentration of bone samples of some wild animals. The results are mean ± SD of 10 analyses of each sample collected from different sources at different times. $P = 0.5$

Metals (µg/g)	*Felis bengalensis*	*Panthera pardus*	*Vivera zebitha*	*Petaurista magnificus*
Lead	22.5 ± 4	ND	3.9 ± 0.2	8.4 ± 0.5
Chromium	21.1 ± 5	ND	ND	ND
Mercury	*32.2 ± 5*	*18.3 ± 3*	*25 ± 5*	*14.2 ± 3*
Selenium	8.2 ± 0.4	5.1 ± 0.2	4.5 ± 0.2	8.3 ± 0.5

Table 3. Concentration of some elements in soil and water samples from home range of animals (seasonal variations)

Time of sampling	No. of samples		Cr (µg/g)		Hg (µg/g)		Pb (µ/g)		Se (µ/g)		V (µ/g)	
	soil	water	soil	water	soil	water	soil	water	soil	water	soil	water
January–March	30	25	0.5–3.0 (1.7)	ND	0.5–3.6 (2)	ND	1.2–15 (8.1)	ND	0.8–17 (9.0)	ND	0.4–15 (8)	ND
April–June	25	25	0.4–3 (1.7)	ND	0.6–6.1 (3.3)	0.6–1.3 (0.9)	1.2–16 (5.9)	1–2 (1.5)	0.7–15 (8.0)	0.0–1.2 (1.0)	0.4–16 (8.2)	ND
July–September	30	35	0.5–3 (1.7)	ND	0.6–5.8 (3.2)	0.6–2 (1.3)	1.3–14.5 (7.9)	1–2 (1.5)	0.8–16 (8.2)	0.9–1.4 (1.1)	0.3–16 (8)	ND
October–December	30	30	0.4–3 (1.7)	ND	0.5–3 (1.7)	ND	1.0–13.5 (7.2)	ND	0.7–14 (7.1)	ND	0.3–15 (7.8)	ND

Figures in parentheses denote mean.

various elements involved scanning electron microscopy (JSM-35CF, Jeol) of epidermal surface, hair surface, hair follicles, etc. using conventional methods. Behavioural studies in relation to symptoms associated with toxicity and deficiency of elements were carried out either in a zoo or in the home range of animals.

The following observations were made in relation to different elements in the four animal species studied.

The level of mercury in body hair was found to cross the toxic limit in *Felis bengalensis*, while in *Panthera pardus*, *Vibera zebitha* and *Petaurista magnificus*, the concentration was within the toxic limit, but on the higher side (Table 1). It is to be noted that deposition of Hg in the hair is known to be proportional to that of blood, after the intake of the metal[6]. The concentration of the metal in the bone was also found to be the highest in *Felis bengalensis* compared to the other animals studied (Table 2).

Elemental analysis revealed that quite a high concentration of the metal is present in soil in the home range of the animals (Table 3), while in water, the concentration was found to be high only during rainy season, probably due to leaching from the soil (Table 3). It is worth mentioning that a large deposit of mercury is reported in the Himalayan belt[7] which includes the present study area. However, release of methyl mercury rather than inorganic mercury into the environment has a more direct ecological impact[7]. In Sweden, a striking number of deaths, and decline in the population of seed-eating birds and their predators was due to the use of methyl mercury for seed dressing[7]. The leopard cat, in which mercury was found to be present in toxic concentrations,

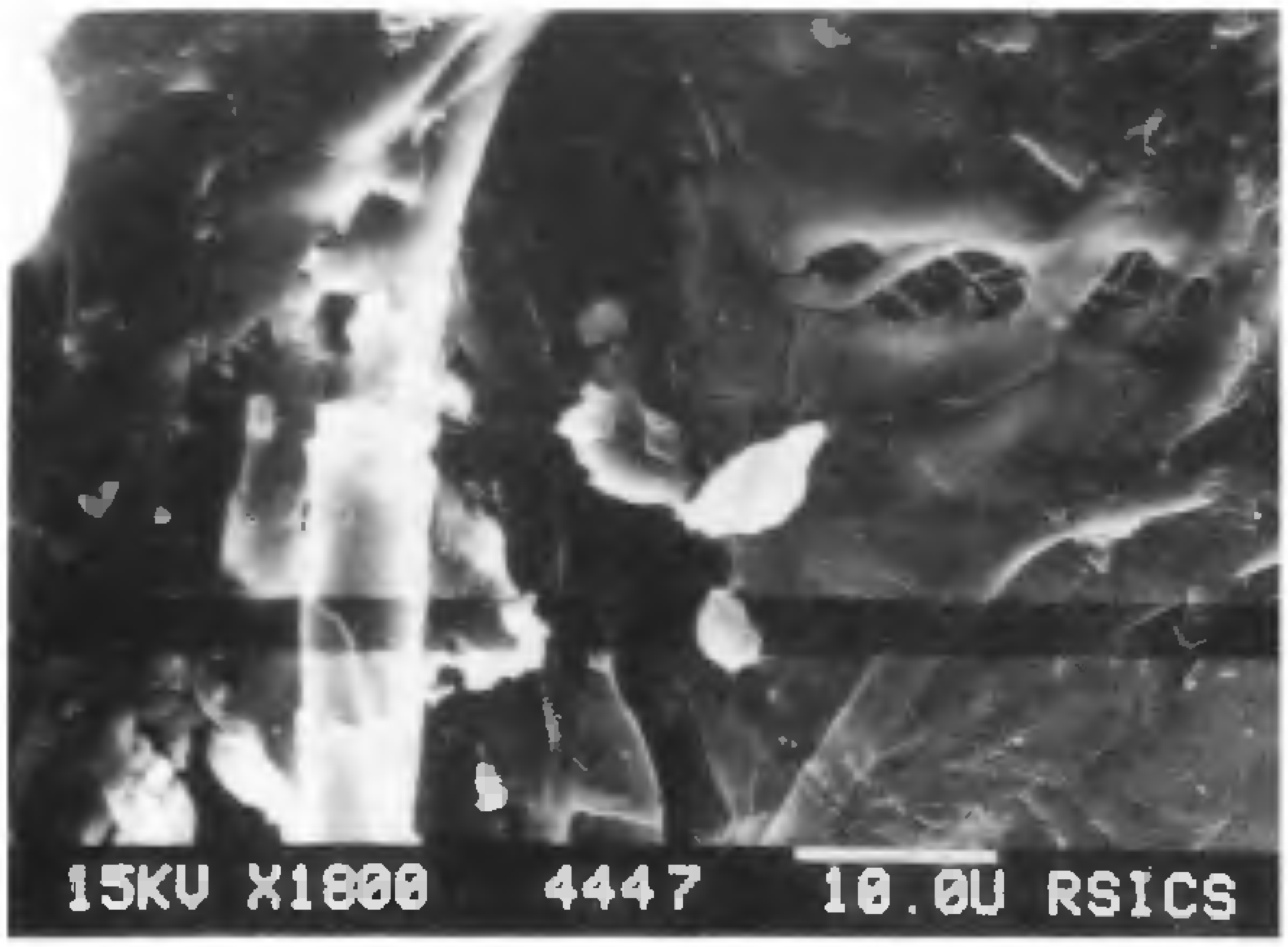

Figure 1. Blister and lesions in the epidermis of the skin in *Felis bengalensis*, as revealed by scanning electron micrograph (bar = 10 μm).

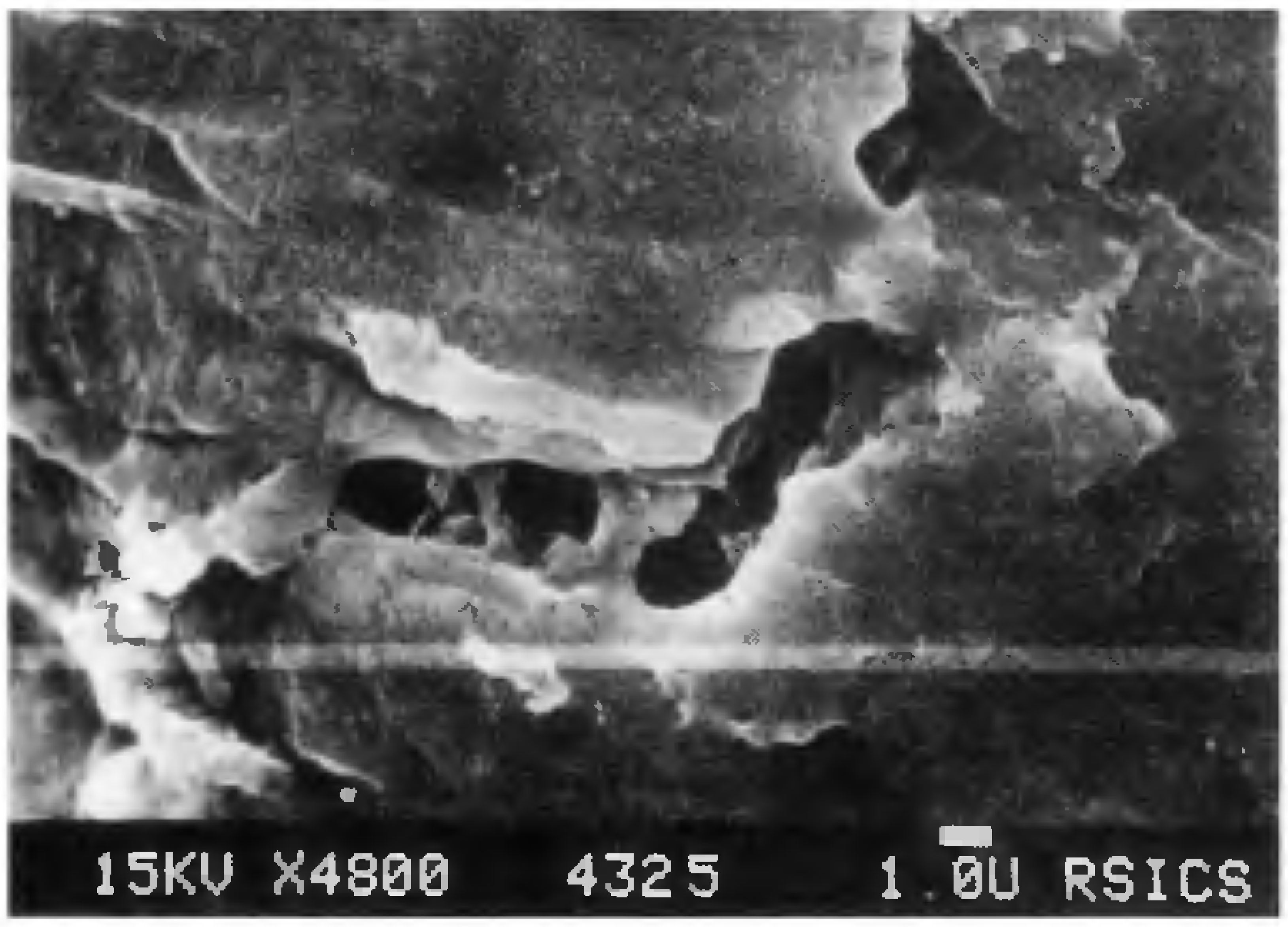

Figure 2. Scanning electron micrograph of epidermis of the skin in *Petaurista magnificus* showing lesions (bar = 10 μm).

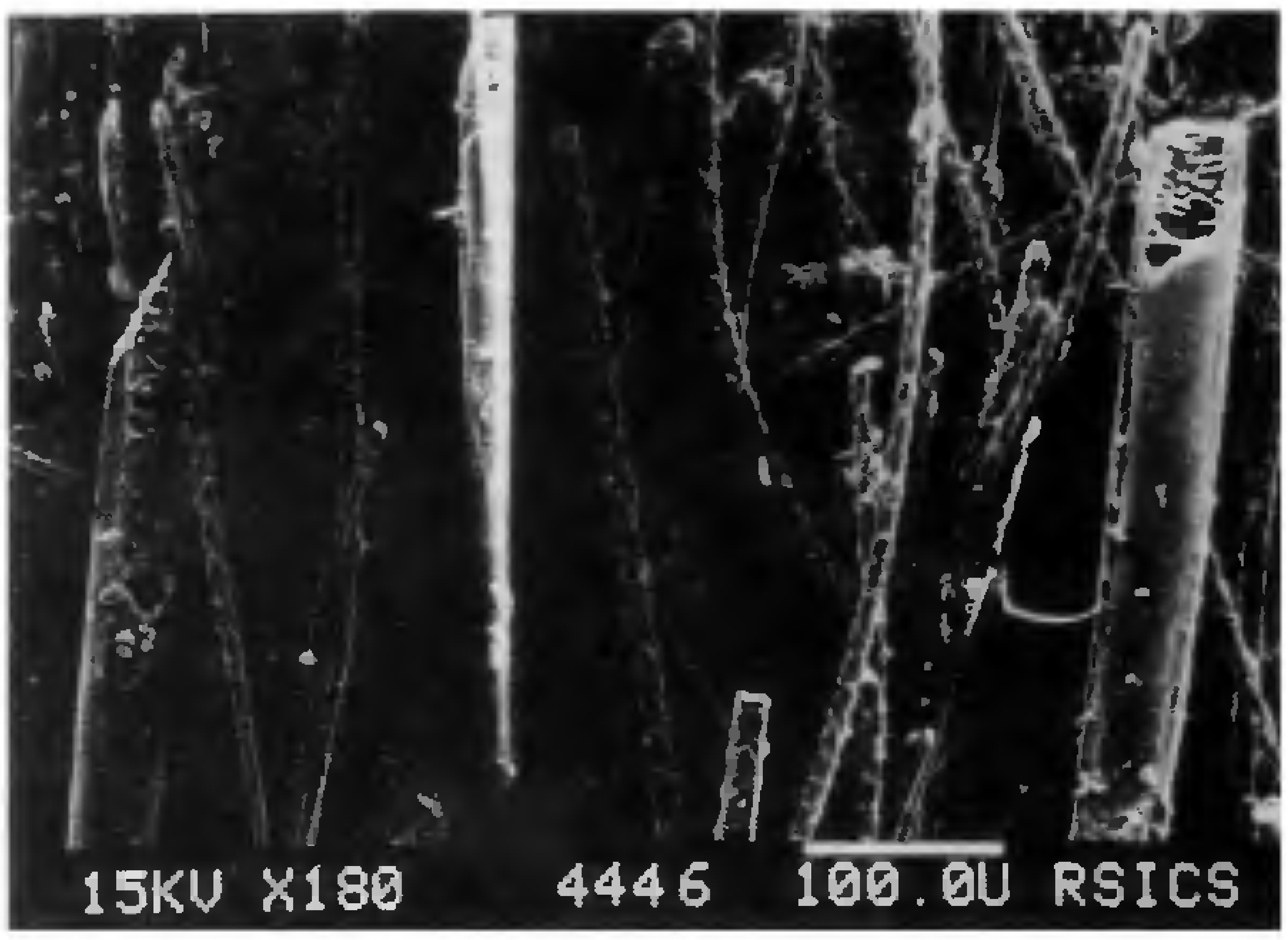

Figure 3. Scanning electron micrograph of body hair of *Felis bengalensis* showing brittleness of hair (bar = 10 μm).

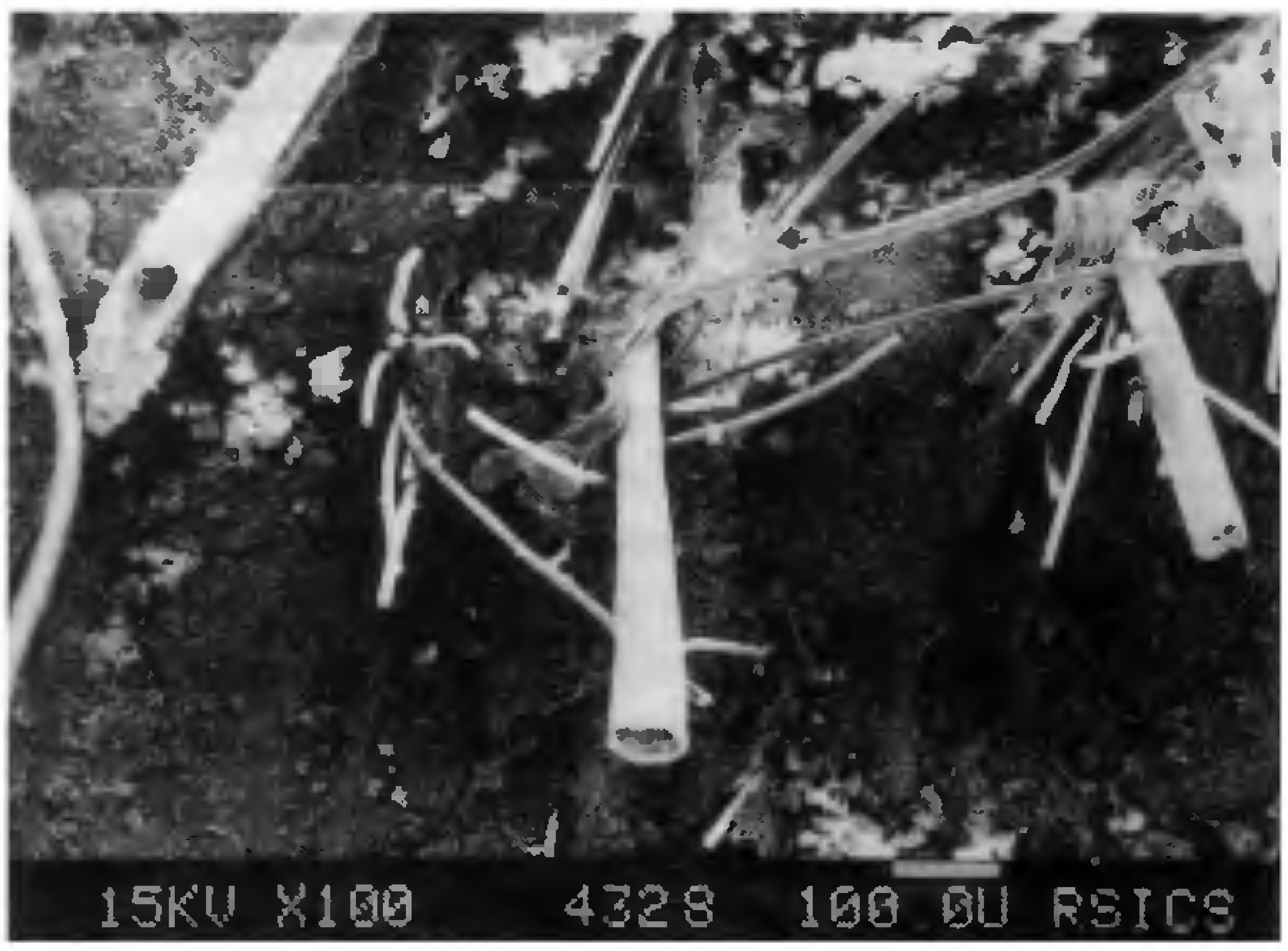

Figure 4. Scanning electron micrograph of brittle hair of *Vivera zebitha* (bar = 100 μm).

preys upon seed-eating birds and small animals[8]. Civet cats and flying squirrels are also reported to be fruit and seed eaters. The leopard also preys upon seed-eating birds, small beasts of prey, large rodents, etc., besides cattle, deer and monkeys[8]. Further, mercury discharge due to caustic and chlorine production; material processing, e.g. live stock manure, tar and asphalt; mining and smelting; burning of fuels, e.g. coal, oil and natural gas appears to be quite high in the areas surrounding the home range of the animals studied.

The toxic effect of mercury was observed only in *Felis bengalensis*, since in other animals, although the concentration of the metal was quite high it was below the toxic limit. Redness of skin, blisters (Figure 1), salivation, loss of appetite, and photophobia, are some of the characteristic toxic effects of mercury[7] observed in the animal.

Lead was detected in the hair and bone of all the animal species, except in the leopard. However, the concentration was above the toxic limit in leopard cat and flying squirrel (Tables 1 and 2) only.

Accumulation of lead in the leopard cat and civet cat is likely to be due to their characteristic behaviour pattern and frequent movements to human territory. The leopard cat and civet cat are known to supplement their food supply by invading human domains to prey on poultry, rat and other vermin flourishing in and around human settlements[8]. Civet cats often live in crowded cities, finding shelter in drains or in roofs of houses[8]. Flying squirrels on the other hand, are essentially forest animals but they sometimes roost under the roofs of

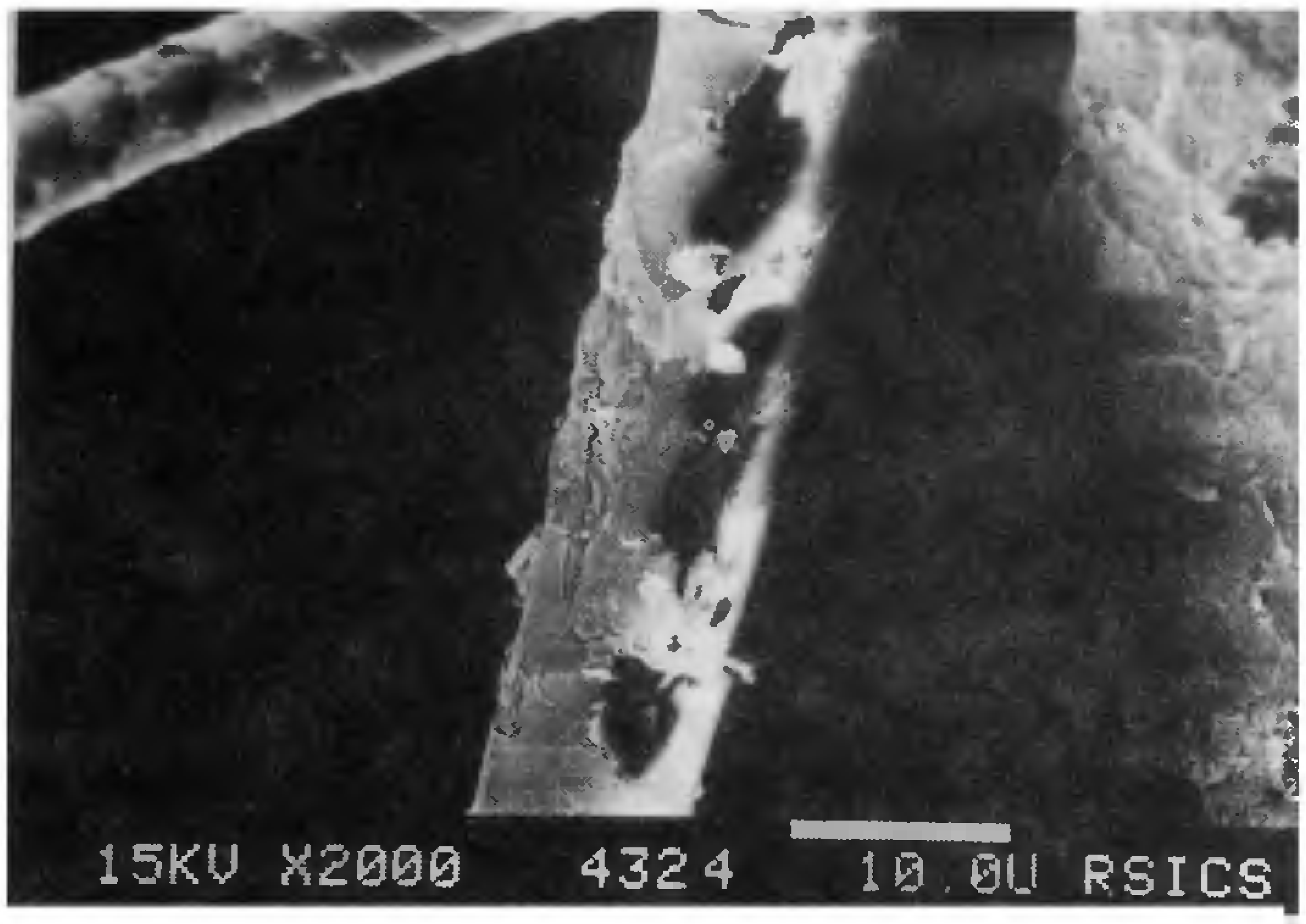

Figure 5. Scanning electron micrograph of brittle hair of *Petaurista magnificus* (bar = 10 μm).

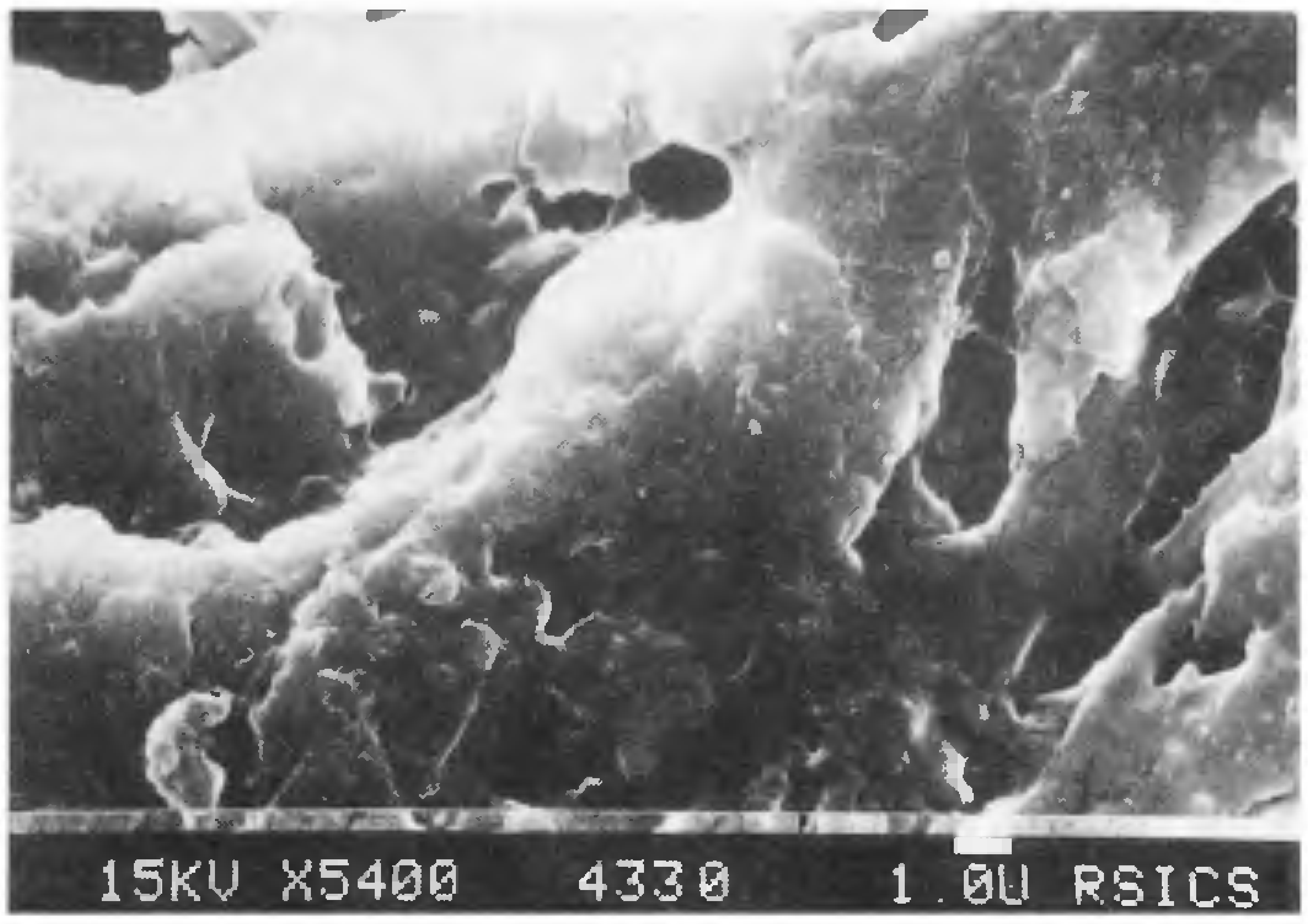

Figure 7. Scanning electron micrograph of epidermis of the skin in *Panthera pardus* showing lesion, eruption and blisters (bar = 1 μm).

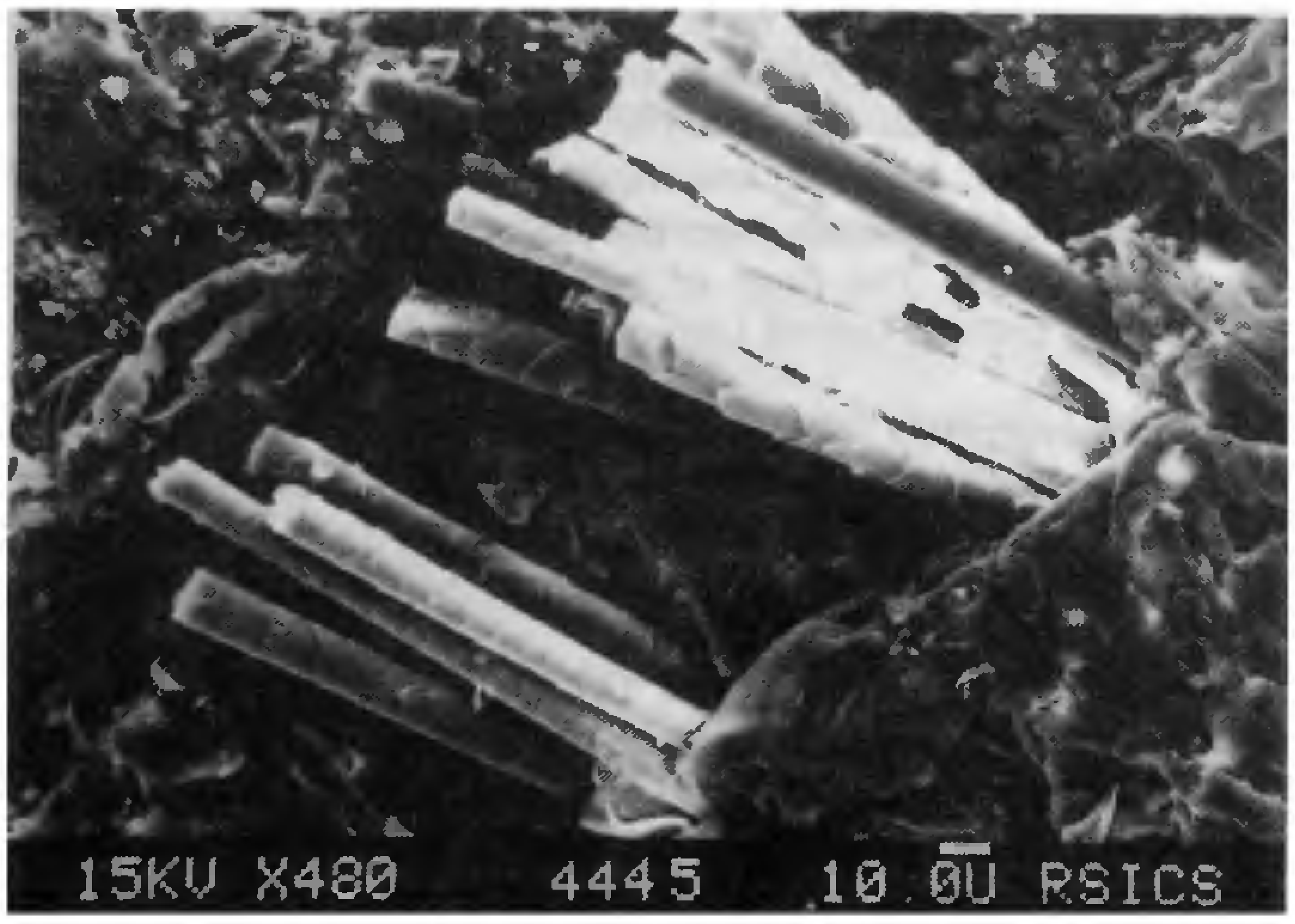

Figure 6. Scanning electron micrograph of brittle hair of *Panthera pardus* (bar = 10 μm).

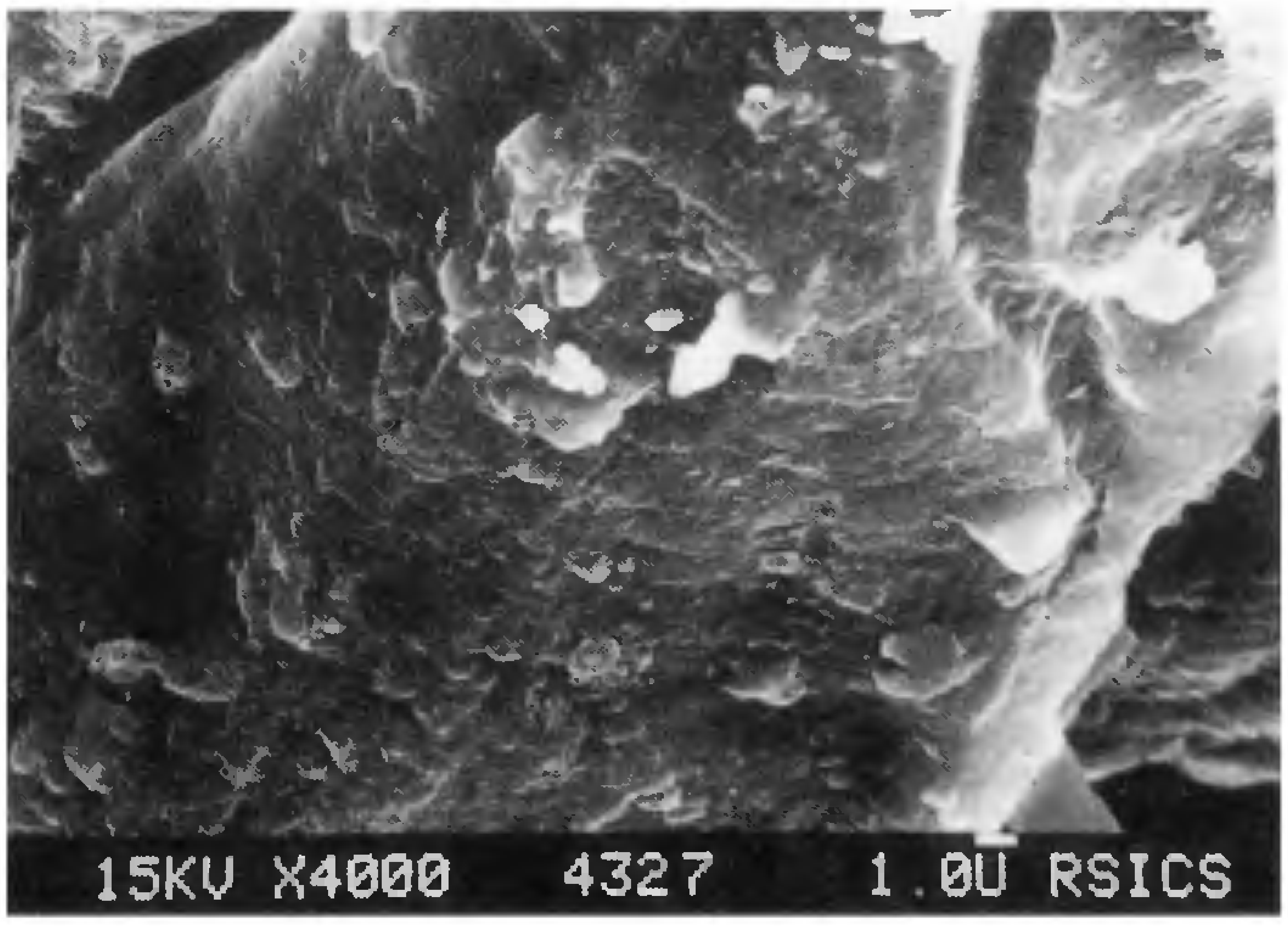

Figure 8. Scanning electron micrograph of the skin in *Petaurista magnificus* showing blisters and eruptions (bar = 1 μm).

forest bungalows. Their food consists of fruits, nuts, insects, and their larvae[8]. Hence, it is difficult to predict the exact source of lead accumulation in their body. The soil in the home range of the animal, with low soil pH and high humus content was also found to contain high concentration of lead. The content of the metal in water, on the other hand, was found to be high only during rainy season (Table 3). The arboreal habit of the animal may also contribute to the exposure and accumulation of air-borne lead. Absence of lead in leopard may be related to its infrequent association with human territory and also to the variation in species response to the uptake of the metal.

The visible toxic effect of lead in *Felis bengalensis* and *Petaurista magnificus* includes lesions in epidermis of skin (Figures 1 and 2), constipation, loss of appetite, etc. Besides these, the metal is known to affect the central nervous system, reproductive system, blood, kidney and liver. Sterility caused due to the accumulation of lead seems to be of significant relevance to the wild life conservation in the present context. Further, the effect of the metal in suppressing some major components of the immune system[7] is likely to have a significant adverse impact on wild animals, since lead, as evidenced from the present study, had made its entry into the wild life ecosystem of the region.

Selenium in the hair has been found to exceed toxic limits in all the four animal species studied (Table 1). The level is also found to be quite high in the bone (Table 2).

Elemental analysis of soil in the home range of the animals revealed that certain areas contain large amount of selenium, while in others, the concentration of the element was low (Table 3). Report on similar observations on the occurrence of local selenium eco-concentration or depletion exists in literature[7]. Selenium often occurs in the earth's crust in association with sulphur-containing minerals (0.05–0.9 μg), volcanic and sedimentary rocks (> 10 μg/g) and fossil deposits such as coal and oil (0.5–10 μg/g or more)[9,10]. Some of the above-mentioned geological conditions for high selenium content in the soil exist in the home range of animals used in the present study. One of the important sources of selenium toxicity in animals is reported to be certain plants[7], including cereals, which are known to accumulate high levels of selenium in non-protein amino acids[11]. This suggests the possibility of occurrence of some selenium-accumulating plants in the region.

Certain known toxic effects of selenium, such as brittleness of hair[7] (Figures 3–6), appearance of blisters and erruptions in the skin[7] (Figures 1, 2, 7, 8), loss of long hair[7], loss of appetite[7] and tendency to wander in a circle[7] were observed in several individuals of all the four animal species studied. Besides these, liver cirrhosis, impaired vision, mutagenic and carcinogenic effects of selenium compounds have been observed in experimental animals[12,13] by several authors.

Chromium could not be detected in the bone and body hair of *Panthera pardus, Vivera zebitha* and *Petaurista magnificus* (Tables 1 and 2) indicating a severe deficiency of the element in these animals. Report on chromium deficiency in human and animals, presumably due to inadequate intake, poor availability and disturbances in uptake of the element exists in literature[14]. The chromium concentration in the blood is not suitable for diagnosis of chromium supply status, and hence chromium concentration in the hair is regarded as an useful indicator for diagnosing the status of the element in the body[15]. The normal chromium status observed in *Felis bengalensis* suggests that the uptake of the element is not disturbed in the animal. This indicates that variation in species response to disturbances in chromium uptake may exist.

It is known that the uptake of chromium is inhibited by vanadium[16], which was found to have a higher concentration than chromium in the soil of the home range of the animals (Table 3). Report on the presence of higher concentration of vanadium when compared to chromium in soil of certain areas of north-east India exists in literature[17]. Vanadium content of the environment is linked with petroleum combustion[18], burning of coal[19], and dumping of solid industrial wastes[20]. The presence of a huge coal deposit in Meghalaya and oil field in Assam may contribute to the high vanadium level of the soil of the region.

Deficiency of chromium leads to impaired glucose tolerance and weight loss[7], which is likely to have a significant impact on the wild life population.

The observations made in the present study suggest that toxicity of certain elements in some wild animals of north-east India is due to environmental pollution or natural abundance of some heavy metals. Similarly, deficiency of certain trace elements due to poor uptake results from the inhibitory effect of some heavy metals, predominant in the region because of geological conditions and human activity. The study further suggests that current wild life conservation management strategies in north-east India will remain incomplete without proper attention to the elemental status of animals and their environment.

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Received 3 April 1998; revised accepted 1 April 1999