

scope to trace the routes of infection.

The developmental stages of *B. bassiana* are the conidium, vegetative and aerial hyphae. The conidia are oval or spherical shaped (Figure 1 a, c, e). Each conidium germinates within 8–10 h upon contamination of host integument<sup>4</sup>. The conidia put forth a germ tube which releases chitinase, facilitating penetration of the host integument and haemolymph<sup>1,4</sup>, succeeded by development of several small vegetative hyphae (Figure 1 b, d) which depletes the nutrient and water content of silkworm. The vegetative hyphae also produce toxins causing intoxication and death<sup>4</sup>. After the seventh day of inoculation a large number of vegetative hyphae emerge from the integument to form aerial hyphae, developing into several conidiophores, which give rise to small branches, each of which bear one or more oval or spherical conidia (Figure 1 c, e) similar to the earlier reports<sup>3,4</sup>. The conidiophores emerge from the base of septum of aerial hyphae (Figure 1 c, e). Each conidium subsequently detaches (Figure 1 e) by osmotic pressure. A large number of conidia on host integument gives a whitish appearance. The multi-layered crystals of ammonium and magnesium oxalate have been observed on the integument of the infected larvae (Figure 1 f) and in haemolymph. The crystals are of different sizes and shapes and considered to be the byproducts of infection. However, the formation and function of

such crystals are not known. The severely infected larva becomes stiff and whitish in appearance because of heavy deposition of conidia and crystals. Generally the infection begins soon after the silkworm integument becomes contaminated with mature conidia. The route of infection through integument in silkworm has been reported by earlier workers based on visual and light microscope observations<sup>1,2,4</sup>, whereas in the present study we have observed the route of infection under scanning electron microscope which has given a firm support to the earlier reports. Moreover, the oral inoculation of conidia has also been reported causing infection<sup>5,6</sup>, and sometime infection may also occur through tracheal openings and digestive tract in *B. mori*<sup>1</sup>.

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## Analysis of trace elements of some edible trematodes parasitizing the bovine hosts

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With the aid of atomic absorption spectrophotometer a qualitative and quantitative analysis of trace element composition of some edible trematode parasites namely, *Gastrothylax crumenifer*, *Fischoederius elongatus*, *F. cobboldi*, *Calicophoron calicophorum*, *Orthocoelium orthocoelium* and *Paramphistomum epiclitum* revealed the occurrence of Cu, Ca, Mg, Mn, Pb, Fe, Ni, Zn, Cr, Cd, K, Se and Co in all the studied species, with K showing the highest concentration and Co the lowest in dry weight of the flukes. Further, Ca, Fe, Zn, Cr and Se were found to be higher in immature and Cu, Mg, Mn, Cd, K and Co were more in mature *G. crumenifer* and *F. elongatus*.

TRACE elements, which are not synthesized in the animal tissue but have significant role in the normal functioning

of the body, constitute an important diet among vital foods. However, both excess and deficiency of any one of these metals may lead to toxicity and metabolic, reproductive and skeletal disorders in the body<sup>1</sup>.

Among helminth parasites, the paramphistomid flukes recovered commonly and in abundance from the rumen of cattle and buffaloes constitute an unusual food item and a non-traditional source of animal protein relished by the local tribal population of Meghalaya. In context of helminth parasites, trace elements of several cestode and nematode species have been investigated<sup>2–5</sup> and their content found to be species-specific<sup>6</sup>. However, similar information with regard to trematode parasites is relatively scanty<sup>7,8</sup>. The present communication deals with a qualitative and quantitative analysis of trace elements of the edible trematodes all of which are amphistomid digenea.

Live parasites, namely *Gastrothylax crumenifer*, *Fischoederius elongatus*, *F. cobboldi*, *Calicophoron calicophorum*, *Orthocoelium orthocoelium* and *Paramphistomum epiclitum* were recovered from the rumen of cattle, *Bos indicus*, slaughtered at local abattoirs. The mature specimens of all the six species and also the immature (i.e.

without eggs in the uterus) of *G. crumenifer* and *F. elongatus* were washed thoroughly in double distilled water and dried at 100°C for 24 h in an oven. The dried material was ground and 0.5 g of ash from each sample was digested in 5 ml of 2 N nitric acid for 12 h followed by evaporation of the acid at 50°C (ref. 9). This process of digestion was repeated thrice. About 2 ml of HClO<sub>3</sub> was then added to the dried sample and the final volume made to 50 ml. Cu, Ca, Mg, Mn, Pb, Fe, Ni, Zn, Cr, Cd, K, Se and Co was estimated by atomic absorption spectrophotometer (238A).

The atomic absorption spectra of mature and immature *G. Crumenifer* and *F. elongatus* and mature specimens of *F. cobboldi*, *C. calicophorum*, *O. orthocoelium* and *P. epiclitum* indicated the presence of all the trace elements assayed for (Table 1). The overall accumulation of trace metals was found to be highest in *F. cobboldi* followed by *F. elongatus*, *P. epiclitum*, *C. calicophorum*, *G. crumenifer* and *O. orthocoelium*. The amounts of K, Mg, Ca, Fe and Zn were found to be more in all the flukes examined, whereas Co and Cd were in least amount in most of the worms, with the lowest values being in *F. elongatus* and *P. epiclitum*. The results reveal that the quantity of Ca, Fe, Zn, Cr, and Se was higher in immature than mature flukes, while Cu, Mg, Mn, Cd, K and Co were more in mature worms. The values of Pb and Ni do not show any relationship with the maturity of the fluke; while Pb was high in concentration in immature *G. crumenifer* and mature *F. elongatus*, Ni was maximum in mature *G. crumenifer* and immature *F. elongatus*.

High values of K among all the flukes may be accounted for the possible flux of K<sup>+</sup> between the host and parasite, since helminths are known to show permeability to K<sup>+</sup> ions<sup>10</sup>. Similar to the present observations, Yamane *et al.*<sup>3</sup> observed a tendency of Ca, Fe and Zn

to increase from immature to gravid proglottid in the cestodes *Diphyllbothrium macrovatum* and *Diplogonoporus balaenopterae*. They suggested that Zn is essential for growth and as a constituent of enzymes, DNA, RNA and protein synthesis. Chowdhury and Singh<sup>4</sup> also revealed a high level of Zn in growing flukes with active oogenesis and spermatogenesis than the older ones.

Cadmium, though occurring in less amount among all the flukes studied, is known to be very toxic to man as it accumulates in kidney and also affects the reproductive organs. (According to WHO<sup>11</sup>, the acceptable limit in drinking water is 0.01 ppm.) Likewise, Pb with its permissible intake limit being 0.1 ppm (ref. 11) may also become hazardous if consumed 2–4 ppm over a period of three months. Selenium, an essential element for growth in animals, was also detected in all the edible flukes. An over supply of Se may lead to dullness and lack of vitality to an acute form<sup>1</sup>. Comparatively a high level of Co, an integral part of vitamin B<sub>12</sub> molecule, in mature worms indicates the probability of high accumulation of vitamin B<sub>12</sub> in adult flukes. Excess intake of Co and Mn is also hazardous to human health with implications of cardiac and neurological disorders, inflammation of cells and also inhibition of the key enzymes of glycolysis<sup>12</sup>.

When compared with the commercially important traditional fish food like *Sardinella* spp., *Thryssa* spp. and *Stolephorous* spp., the quantity of Fe, Zn and Mn was found to be much more in edible helminths. Sivakumar *et al.*<sup>13</sup> reported the occurrence of Fe, 68–110 ppm; Zn, 38–70 ppm; Cu, 20–33 ppm and Mn, 1–22 ppm in dry weight of these fishes.

It is thus evident that these helminth parasites provide a ready source of nutrients required for our normal physiology. However, the occurrence of a considerable

Table 1. Trace element content in different species of edible paramphistomid flukes (Mean  $\pm$  SD: ppm/g dry wt)

	Cu	Ca	Mg	Mn	Pb	Fe	Ni	Zn	Cr	Cd	K	Se	Co
<i>G. crumenifer</i> (Immature)	14.6 $\pm 5.1$	122.8 $\pm 29.5$	353.0 $\pm 148.5$	6.6 $\pm 3.0$	36.7 $\pm 17.9$	127.1 $\pm 10.1$	8.7 $\pm 2.8$	182.2 $\pm 47.7$	48.1 $\pm 15.8$	1.5 $\pm 0.8$	2185.0 $\pm 346.8$	15.5 $\pm 5.2$	2.5 $\pm 1.2$
<i>G. crumenifer</i> (Mature)	19.9 $\pm 14.3$	100.2 $\pm 28.6$	439.7 $\pm 240.5$	47.9 $\pm 9.2$	4.1 $\pm 1.9$	108.6 $\pm 27.8$	26.4 $\pm 7.7$	42.2 $\pm 14.4$	23.7 $\pm 4.3$	13.6 $\pm 7.3$	2242.0 $\pm 880.8$	7.6 $\pm 2.7$	4.1 $\pm 1.9$
<i>F. elongatus</i> (Immature)	24.7 $\pm 11.2$	164.3 $\pm 52.5$	440.0 $\pm 118.0$	15.8 $\pm 5.6$	14.5 $\pm 10.5$	221.7 $\pm 88.4$	46.8 $\pm 12.6$	154.6 $\pm 91.3$	45.3 $\pm 3.9$	0.9 $\pm 0.4$	1943.0 $\pm 840.0$	9.5 $\pm 4.0$	1.0 $\pm 0.3$
<i>F. elongatus</i> (Mature)	88.5 $\pm 24.3$	94.4 $\pm 11.5$	993.4 $\pm 359.2$	20.3 $\pm 8.7$	24.0 $\pm 12.0$	185.9 $\pm 23.9$	13.1 $\pm 7.8$	73.5 $\pm 9.5$	25.7 $\pm 13.6$	14.6 $\pm 4.3$	3630.2 $\pm 497.8$	5.5 $\pm 2.6$	3.0 $\pm 1.7$
<i>F. cobboldi</i>	22.6 $\pm 8.8$	404.0 $\pm 86.0$	185.3 $\pm 55.0$	50.9 $\pm 12.7$	9.7 $\pm 5.2$	169.1 $\pm 36.2$	52.3 $\pm 19.7$	185.0 $\pm 49.0$	64.9 $\pm 14.4$	10.8 $\pm 3.8$	3448.0 $\pm 377.0$	17.2 $\pm 3.0$	5.3 $\pm 2.8$
<i>C. calicophorum</i>	22.1 $\pm 6.8$	141.0 $\pm 28.0$	399.0 $\pm 241.0$	181.8 $\pm 46.5$	17.0 $\pm 9.5$	109.6 $\pm 18.4$	35.9 $\pm 12.8$	274.0 $\pm 73.0$	37.1 $\pm 11.0$	32.9 $\pm 5.6$	2478.0 $\pm 540.0$	16.4 $\pm 5.9$	3.4 $\pm 1.4$
<i>O. orthocoelium</i>	43.3 $\pm 34.4$	82.1 $\pm 41.7$	292.8 $\pm 152.4$	24.8 $\pm 16.5$	12.3 $\pm 6.9$	154.1 $\pm 41.9$	15.3 $\pm 9.3$	122.4 $\pm 45.4$	57.0 $\pm 32.7$	4.5 $\pm 1.3$	1887.9 $\pm 633.9$	59.5 $\pm 10.9$	7.6 $\pm 5.6$
<i>P. epiclitum</i>	15.2 $\pm 3.6$	139.1 $\pm 25.0$	607.0 $\pm 103.7$	20.7 $\pm 4.6$	7.2 $\pm 2.5$	108.0 $\pm 29.2$	17.8 $\pm 3.1$	48.2 $\pm 19.4$	49.1 $\pm 14.7$	9.6 $\pm 4.2$	2771.4 $\pm 491.7$	6.5 $\pm 2.2$	1.4 $\pm 0.9$



amount of Cd and Pb in mature flukes of all the species analysed leads to think about the possible health hazard to the consumers of these parasites.

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