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CYTOPATHOLOGICAL CHANGES IN ERYTHROCYTES OF THE CAT-FISH *HETEROPNEUSTES FOSSILIS* (BLOCH) EXPOSED TO TEXTILE-MILL EFFLUENT

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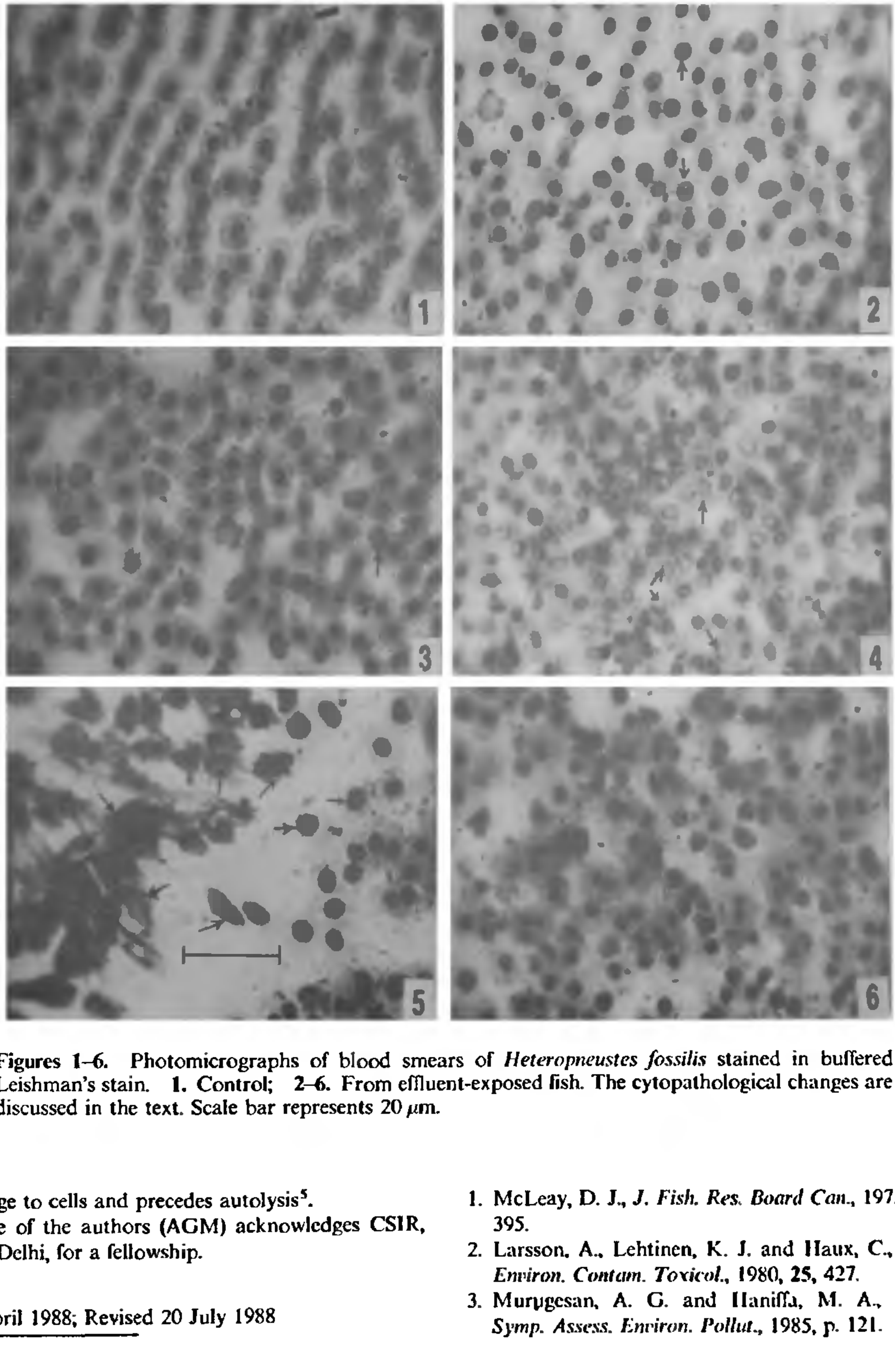
DESPITE the existence of considerable literature on the haematology of organisms exposed to industrial effluents[1-4], there is paucity of information on cytopathological changes brought about in blood cells by effluents. This paper attempts to fill the gap. In the course of a haematological study of the freshwater cat-fish, *Heteropneustes fossilis*, exposed to sublethal concentrations of partially treated textile-mill effluent, the authors were struck by the series of changes undergone by the erythrocytes of the exposed fish. The effluents discharged by the textile-mill into the river Tambaraparni are composed of several cations such as sodium, ammonium, magnesium, copper, chromium and zinc, besides potassium and calcium, and anions such as sulphate and chlorides, as well as nitrates and nitrites. The levels of these ions exceed the safe limits prescribed by the government. The authors believe that the haematological changes in the exposed cat-fish are due to the synergistic effect of the several components of the complex of ions present in the effluent.

Healthy *H. fossilis* of 25 ± 3 g live-weight were acclimated to laboratory conditions by feeding them on beef slices *ad libitum* for a fortnight. The combined textile-mill effluent used was obtained from Madura Coats textile mill, Papanasam, and consisted of dyeing effluent, bleaching effluent and kiering effluent. The pH of the dyeing effluent was 11.2, that of bleaching effluent 11.9, and that of kiering effluent 11.7; the combined effluent had a pH of 6.9. The textile mill releases the combined effluent into the river after partial treatment. The LC_{50} value was determined, and sublethal concentrations such as 2, 3, 6 and 9% were prepared using dechlorinated tap-water. The fish were reared in the treated water for 120 days. A control, using tap-water, was also maintained simultaneously. After the period of exposure, the caudal fin was severed to get the blood for smearing. Buffered Leishman's stain of pH 6.8 gave excellent preparations of blood smears (fixed in methanol and then air-dried). The work reported here is based on the analysis of slides of fishes treated with 9% effluent concentration, as the observed changes were maximum in these fishes.

In the control (figure 1) the erythrocytes are elliptical, with oval nuclei containing dense chromatin, stained intensely; the chromatin appears granular and basiphil; the cytoplasm does not show any vacuolation. The erythrocytes undergo distinct changes in the treated fish. An early effect of the effluent is change in their appearance; the erythrocytes are swollen and subspherical. The nucleus is enlarged and is circular in outline (figure 2). The chromatin is dispersed and has a fragmented appearance (karyorrhexis); the chromatin is faintly stained with gaps between the granules (figure 3). Further changes lead to the formation of a large, vacuolated, very faintly stained nucleus (figure 4). In some erythrocytes, only the nuclei are visible in the smear, there being no cytoplasm surrounding them. This is because the hypertrophied erythrocytes have weak cell membranes which break during the process of smearing, releasing the nuclei (figure 5).

In the pathological specimens the nuclei are large, flattened discs, stained pinkish (instead of bluish purple). In extreme cases the nuclei take a lobed appearance (figure 5). The sequence illustrated here shows the series of cytopathological changes of the erythrocytes caused by exposure of the fish to the textile-mill effluent. Figure 6 shows some of the conspicuous changes.

Preliminary observations reveal that the cytoplasm of the leucocytes also shows vacuolation as a result of treatment with textile-mill effluent. It may be mentioned that vacuolation is the earliest sign of



Figures 1–6. Photomicrographs of blood smears of *Heteropneustes fossilis* stained in buffered Leishman's stain. **1. Control; 2–6.** From effluent-exposed fish. The cytopathological changes are discussed in the text. Scale bar represents 20 μm.

damage to cells and precedes autolysis[5].

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DEMORPHOGENESIS OF MUSTARD APHID, *LIPAPHIS ERYSIMI* (KALT.) BY THE VAPOURS OF JUVENILE HORMONE ANALOGUES

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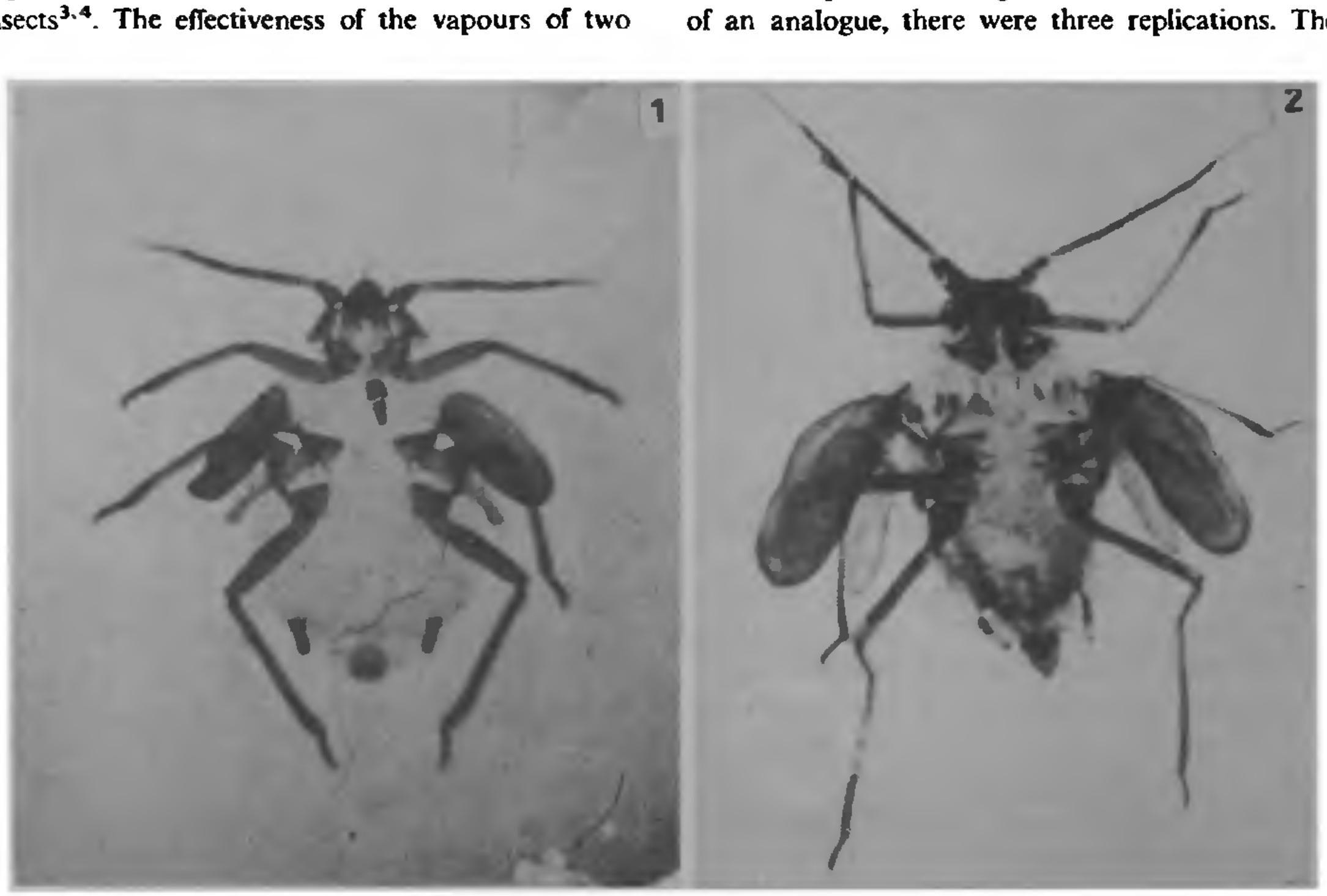
THE application of juvenile hormone analogues (JHAs) to immature insects imparts demorphogenesis and the hemimetabolous insects at the penultimate-instar level are greatly affected by these chemicals[1,2]. However, information regarding the effectiveness of vapours of JHAs is available only for a few insects[3,4]. The effectiveness of the vapours of two juvenile hormone analogues, R-20458 (6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene) and ZR-512 (ethyl 3,7,11-trimethyl-2,4-dodecadienoate) to impart demorphogenesis to the mustard aphid, *Lipaphis erysimi* (Kalt.) exposed at the penultimate-instar level to the JHAs is reported.

The mustard aphid, *Lipaphis erysimi* (Kalt.) was reared on radish plants, *Raphanus sativus* L., grown in pots under cage conditions. Third-instar aphids (presumptive alatae) bearing miniature green wing buds were selected for the experiment. Filter paper discs were fixed to the bottom of crystallizing dishes (50 mm × 100 mm) and 0.5 ml of each concentration, (0.001, 0.01, 0.1 and 1%) of the JHAs(R-20458 and ZR-512) in acetone was applied to each disc using a one ml pipette. The filter paper discs under control were treated with 0.5 ml acetone per disc. A radish leaf with 25 to 30 third-instar aphids (presumptive alatae) was kept on the moist filter paper lying at the bottom of petri dish (15 × 155 mm). After 30 min of the treatment of filter paper discs, the crystallizing dishes were inverted over the leaves supporting test-insects so that their bottoms turned upwards to make the operation air tight. For each concentration of an analogue, there were three replications. The



Figures 1 and 2. **1.** Normal fourth instar (presumptive alatae) of mustard aphid, *Lipaphis crysimi* (Kalt.), and **2.** Nymphal-adult intermediate of mustard aphid, *Lipaphis crysimi* (Kalt.).