List of Figures

Figure 1. Evolutionary time scale of Phylum Mollusca.

Figure 2. Natural habitat of *B. bengalensis* (A); association of *B. bengalensis* with freshwater sponge *Eunapius carteri* (B); ventral (C) and dorsal (D) views of the specimen; live specimens of *B. bengalensis* are being attached on submerged rocky substratum (E) and decaying leaf of coconut tree (F); live specimens are being sold in the local market for consumption by human and poultry (G, H).

Figure 3. Developmental stages of *B. bengalensis* in brief.

Figure 4. Anhydrous sodium carbonate

Figure 5. Commercial brand of detergent powder acts as a major contaminant of the freshwater ponds and lakes. Detergent in plastic bags like this one are often kept near the ponds for ready use (A); large water body situated near the eastern metropolitan bypass of Kolkata metropolis is being perennially contaminated by washing soda during washing of clothes by the professional washermen or ‘dhobis’. More than fifty numbers of stone slabs (↑) have been identified in a single pond which are regularly used as platforms of washing clothes (B). Washing soda is a corrosive toxin and acts as an irritant to many living organisms including human, professional washermen often wrap their legs with polythene sheet to avoid washing soda exposure during washing (C). Dead and decaying specimens of *B. bengalensis* (↑) lying along the mud water interface of their habitat which heavily contaminated with washing soda due to laundry activity. These specimens were assumed to be the victims of the acute toxicity of washing soda. Several empty plastic bags of washing soda (↑↑) like this one have been found in the same pond site (D).

Figure 6. Several brands of washing soda are being sold in the commercial outlets of rural and urban West Bengal (A, B); washing soda is an odourless, powdery and white chemical compound (C); contamination of the natural habitat of *B. bengalensis* takes place during cleaning of utensils (D), bathing of human and washing of clothes (E) by washing soda.

Figure 7. a. Toxicity of washing soda in *B. bengalensis* with reference to the functional attribute of hemocytes.

Figure 8. Generation of antimicrobial reactive oxygen and nitrogen species.

Figure 9. A scheme for phenoloxidase activation in invertebrates.

Figure 10. Manual collection of *B. bengalensis* from pond (A); ‘leaf trapping method’ of collection of the specimens (B); *B. bengalensis* distributed along the mud-water interface of pond (C, D); specimens were being stored and acclimated in controlled laboratory condition (E, F).

Figure 11. Collection of hemolymph and isolation of hemocytes of *B. bengalensis*

Figure 12. Flow cytometric analyses of hemocytes of *B. bengalensis* is in progress at Center for Research in Nanoscience and Nanotechnology of the University of Calcutta.

Figure 13. Light micrograph of hemocytes exhibiting positive (arrow) and negative response to trypan blue staining. Scale: 10µm.
**Figure 14.** Phase contrast microscopic images of live hemocytes of *B. bengalensis* like blast like cells (A, F); granulocytes (B, G); agranulocytes (C, H); astocytes (D, I) and hyalinocytes (E, J) (magnification: x1000 ). A - E represent control hemocytes of *B. bengalensis* ; F – J exhibit damaged hemocytes of *B. bengalensis* treated with washing soda (5000 ppm for 15 days). Washing soda treatment yielded blebbing (bl) and vacuolation (v) in cytoplasm (n=10; Scale: 10µm).

**Figure 15.** Bright field microscopic images of *B. bengalensis* hemocyte subpopulations stained with Giemsa’s stain. A –E represent the control hemocytes of *B. bengalensis* ; F – J exhibit signs of damage like vacuolation (v), nuclear disintegration (nd), cytoplasmic blebbing (bl) and cytoplasmic disintegration (cd) in the hemocytes of *B. bengalensis* treated with washing soda (5000 ppm for 15 days). (Magnification: x1000). (n=10; Scale: 10µm).

**Figure 16.** Scanning electron micrographs of hemocytes subpopulations of *B. bengalensis*. A, B represent the control hemocytes of *B. bengalensis*; C, D exhibit morphological alteration in the hemocytes of *B. bengalensis* treated with washing soda (5000 ppm for 7 days). n=10.

**Figure 17.** Total count of hemocytes of *B. bengalensis* against 700, 1500, 3000 and 5000 ppm exposure of washing soda for 24, 48, 72, 96 hours and 7 and 15 days of exposure. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 18.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 24 hours. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 19.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 48 hours. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 20.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 72 hours. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 21.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 96 hours. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 22.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 7 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 23.** Dynamics of hemocyte subpopulations of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of detergent washing soda for 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 24.** Flow cytometry and phase contrast microscopy (x1000) of hemocyte morphotypes of *B. bengalensis* (a-h). P1 gate represent agranulocytes (a, b, c), P2 gate represents semigranulocytes (d, e) and P3 gate represents granulocytes (f, g, h). The dot-plot presentation of hemocytes by flow cytometry is representative of one experiment over 10 determinations. The observation of live morphotypes under phase contrast microscope was performed over 10 times. n=10. Scale: 10µm.

**Figure 25.** Morphological subpopulations of molluscan hemocytes as revealed by FACS sorting and microscopy.
**Figure 26.** Morphological subpopulations of sorted hemocyte morphotypes (control and treated) of *B. bengalensis* observed under microscope (1000X) after staining with Giemsa’s stain. Agranular subpopulations included blast like cells (a, i) round hyalinocytes (b, j) and spindle hyalinocytes (c, k); semigranular subpopulations included asterocytes (d, l), round semigranulocytes (e, m); granular subpopulations included round granulocytes (f, n), spindle granulocytes (g, o) and granular asterocytes (h/p). a – h represent untreated hemocytes of *B. bengalensis* and i – p represent damaged hemocytes of *B. bengalensis* (5000 ppm/15d). n=10, Scale: 10 μm.

**Figure 27.** Photomicrographs of adherent hemocytes of *B. bengalensis* on glass surface under phase contrast (A, D), bright field (B, E), and scanning electron microscopes (C, F). A – C represent control hemocytes; D – F represent washing soda treated (5000 ppm/15days) hemocytes. Scale: 10 μm.

**Figure 28.** Alteration of adhesion efficacy of *B. bengalensis* hemocytes on glass surface under the exposure of 700 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 29.** Images of aggregating hemocytes of *B. bengalensis* under phase contrast optics (A, D), bright field optics (B, E), and scanning electron microscope (C, F). A – C represent control hemocytes and D – F exhibit washing soda treated hemocytes (5000 ppm/15 days). Scale:10 μm.

**Figure 30.** Percentage of aggregated hemocytes of *B. bengalensis* under the exposure of 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 31.** Phagocytic response of hemocytes challenged with yeast (Y) as observed under phase contrast (A, D); bright field (B, E) and scanning electron microscopes (C, F). A – C represent untreated hemocytes and D – F represent treated hemocytes (5000 ppm/15 days). Scale: 10 μm.

**Figure 32.** Phagocytic response of hemocytes of *B. bengalensis* against 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days exposure. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 33.** Superoxide anion generation in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 34.** Nitric oxide generation in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

**Figure 35.** (A) Flow cytometric analyses of hemocyte morphotypes of *B. bengalensis* showing the dot-plot representation along FSC and SSC axes depicting three morphotypes of hemocytes namely agranulocytes (P1), semigranulocytes (P2) and granulocytes (P3). Dot-plot representation with percent positive cells and overlay histogram depict positive reactivity of iNOS antibody with three hemocyte morphotypes of *B. bengalensis* (B=Control; C= Treated with 5000 ppm washing soda/96 hours). P4, P5 and P6 bar show the percent positive cells for iNOS staining. In overlay representation, solid lines represents isotype control and dotted line represents iNOS antibody binding by hemocytes. This flow cytometric data depicts representation of same experiments repeated for at least five times with similar result.
Figure 36. Phenoloxidase activity in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 37. Phenoloxidase activity in the gill of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 38. Phenoloxidase activity in the digestive gland of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 39. Phenoloxidase activity in the mantle of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 40. Catalase activity in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 41. Catalase activity in the gill of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 42. Catalase activity in the digestive gland of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 43. Catalase activity in the mantle of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 44. Superoxide dismutase generation in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 45. Superoxide dismutase generation in the gill of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 46. Superoxide dismutase generation in the digestive gland of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 47. Superoxide dismutase generation in the mantle of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 48. Glutathione -S- transferase activity in the hemocytes of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).
Figure 49. Glutathione -S- transferase activity in the gill of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 50. Glutathione -S- transferase activity in the digestive gland of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 51. Glutathione -S- transferase activity in the mantle of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 52. Acid phosphatase activity in the hemocyte lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 53. Alkaline phosphatase activity in the hemocyte lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 54. GOT activity in the hemocyte lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 55. GOT activity in the gill lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 56. GOT activity in the digestive gland lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 57. GOT activity in the mantle lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 58. GPT activity in the hemocyte lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 59. GPT activity in the gill lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 60. GPT activity in the digestive gland lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).

Figure 61. GPT activity in mantle lysates of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*<0.05).
Figure 62. Retention of neutral red probe in the lysosomes of hemocyte at 0 minute of assay (A); complete diffusion of dye into the cytoplasm is designated as the end point of probe diffusion (B). (Magnification: x1000; Scale: 10 µm).

Figure 63. Neutral red retention time (NRRT) of the hemocytes of B. bengalensis exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 and 15 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (p<0.05).

Figure 64. Flow cytometry of hemocyte morphotypes of B. bengalensis; P1, P2, P3 gates represent agranulocytes, semigranulocytes and granulocytes respectively along FSC and SSC axes (A). Representative dot-plots of annexin-V-FITC and propidium iodide stained hemocyte morphotypes of B. bengalensis exposed to 3000 and 5000 ppm of washing soda for 7 days along with control (B). First, second and third row represent agranulocytes (P1), semigranulocytes (P2) and granulocytes (P3) respectively. Live hemocytes (Q3 quadrant) of the dot plot bear low FITC and low PI fluorescence, apoptotic hemocytes (Q4 quadrant) exhibit high FITC fluorescence with low PI fluorescence. Necrotic hemocytes (Q2 quadrant) exhibited high FITC and high PI fluorescence. Percent parent of apoptotic and necrotic hemocytes (Q4 and Q2 quadrants) were expressed numerically. This flow cytometric data depict representation of same experiment was repeated for at least five times with similar results.

Figure 65. Immunofluorescent detection of apoptosis and necrosis in the hemocytes of B. bengalensis by annexin-V FITC and propidium iodide staining (magnification: x1000). Apoptotic hemocytes generate a relatively intense green (FITC) fluorescence (A) and low red (PI) fluorescence (B) which appears to be yellow upon colocalization (C). Necrotic hemocytes emitted both green fluorescence (D) and intense red fluorescence due to binding of PI to DNA (E). Upon colocalization necrotic hemocytes appeared to be orange (F). The observation of hemocytes under fluorescence microscope was performed for at least 5 times. Scale: 10µm.

Figure 66. Transverse sections of the gill filaments of control B. bengalensis exhibiting elongated lamellae (lm) arranged equidistantly (a, b).

Figure 67. Transverse sections of the gill filaments (c, d) of B. bengalensis exposed to 5000 ppm of washing soda for 7 days exhibiting enlargement of water channel (ewc), dense fibrosis (fr) and infiltration of hyperchromatic anaplastic cells (hac).

Figure 68. Transverse sections of the digestive gland (a, b) of control B. bengalensis exhibiting digestive cells (dc), digestive epithelium (de).

Figure 69. Transverse sections of the digestive gland (c, d) of B. bengalensis exposed to 5000 ppm of washing soda for 7 days exhibiting hyperchromatic anaplastic cells (hac) and inflammatory lysis (ily).

Figure 70. Transverse sections of the mantle (a, b) of control B. bengalensis exhibiting cuboidal epithelium cells (cue) and muscle fibers (mf).

Figure 71. Transverse sections of the mantle (c, d) of B. bengalensis: exposed to 5000 ppm of washing soda for 7 days exhibiting tissue damage.

Figure 72. Relative mobility of B. bengalensis on mud surface (A,B); the arrow indicates the path trailed (pt) by the specimen.

Figure 73. Relative mobility of B. bengalensis exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 days. Data presented as mean ± mean ± SD, n=10. Asterisks indicate the values that are significantly different (p<0.05).
Figure 74. Foot protrusion behaviour of *B. bengalensis* in water (A, B).

Figure 75. Foot protrusion frequency of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).

Figure 76. The aggregation or ‘clumping’ behaviour of *B. bengalensis* (A, B).

Figure 77. Aggregation or ‘clumping’ response of *B. bengalensis* exposed to 700, 1500, 3000 and 5000 ppm of washing soda for 24, 48, 72, 96 hours and 7 days. Data presented as mean ± SD, n=10. Asterisks indicate the values that are significantly different (*p*< 0.05).