- Water comprises two third of Earth's surface. Due to increase in population as well as industrialization, there is ever-increasing pressure on the planet's water resources. The oceans, rivers and other inland water bodies are being "squeezed" by human activities, which further degrade the quality of water. The contaminants in water cause genetic as well as tissue damage in aquatic organisms, which are irreversible and can be transmitted to next generations. The analysis of environmental genotoxicity and histopathology provide useful information regarding the long term effects of these contaminants.
- Due to industrialization and urbanization in Punjab, many industrial units are set up in the state. The wastewater from these industries is discharged into water bodies. Thus, there is tremendous increase in the level of pollution in the rivers of the Punjab. Earlier, the water of rivers Sutlej and Beas is also used for irrigation as well as drinking purposes. Now, sewage, domestic, agricultural wastes and industrial effluents are discharged into river Sutlej at Ludhiana, Jalandhar, Phillaur and Phagwara, which are responsible for water pollution.
- For the present investigation, tannery and paint industrial effluents were undertaken to know their effects on aquatic organisms. These industries were selected because they are major water polluting industries. Tannery effluent is acidic in nature, which contains heavy metals (chromium, hexavalent chromium, sulphide, boron), high amount of TDS, TSS, BOD COD, oil and grease, whereas paint is alkaline, which contain heavy metals (lead, hexavalent chromium, copper, nickel, zinc), high amount of TDS, TSS, BOD COD, phenolics, oil and grease. Tannery industries dump their wastes in kala sanghia drain, which flows into Chitti Bein (rivulet) then joins the river Satluj. Paint industries also discharge their effluents in Buddha nallah that joins the river Satluj at Gorsian, Kedarbaksh in the north-western corner of Ludhiana.
- Among the aquatic organisms, fishes act as bioindicators as they used for monitoring the water quality. They are also excellent model to

analyze genotoxic potential of the aquatic environment because they are constantly in direct contact with water, top in food chain and indirectly affecting the human health. They can be easily cultured in the laboratory to assess the effect and its long term hazards on human health. Presently, *Labeo rohita* is selected because it is abundantly available in the rivers of Punjab, highly sensitive, easily accumulate pollutants, commercially and ecologically important. It can also be easily maintained in laboratory.

- Present work is aimed to evaluate the genotoxic and histopatholoical effect of both the effluents on a freshwater fish, *Labeo rohita*. 96h LC_{50} values of both the effluents were calculated by following the method given by Finney (1971). It came out to be 15.48% for tannery effluent and 31.62% for paint effluent. Two sublethal concentrations based on (1/2 and 1/8 of 96h of LC_{50}) were selected. These were 7.74% and 1.93% for tannery effluent and 15.81% and 3.95% for paint effluent.
- Behavioral responses and morphological changes were recorded after 24h, 48h, 72h, 96h and 120h in control and sublethal concentrations of tannery and paint industrial effluents. These changes are important tool in toxicology as it help to assess the effect of toxicants externally without any test and used as first step in genotoxicity studies. The genotoxicity tests studied were chromosomal aberration test, micronucleus test and erythrocyte aberration test.
- For chromosomal study, slides were prepared from kidney tissue of both control as well as treated fishes (after 24h, 48h, 72h, 96h and 120h) by following the method given by Manna and Sadhukhan (1986). Slides were stained by Giemsa technique as suggested by Tijo and Whang (1965). Somatic metaphase plates of normal and treated groups have been photomicrographed. Individual chromosomes cut from somatic metaphase plates obtained from the control then homologous chromosomes paired on the basis of their length, arm ratio, morphology

and arranged in descending order according to their sizes. Morphometric analysis of chromosomes has been carried out.

- For micronucleus and erythrocyte aberration tests, slides were prepared by using the method of Ayllon *et al.* (2000). An anterior kidney of control as well as treated fishes was gently dragged on pre-cleaned slides for a thin smear and air dried over-night. Slides were fixed in absolute methanol for 14- 20 minutes, air dried for half an hour and stained with Giemsa. Slides of micronuclei formation and erythrocytes Aberrations have been photomicrographed.
- Data of chromosomal aberration test, micronucleus test and erythrocytes Aberrations have been subjected to statistical analysis by applying Annova and Tukey test. Statistical analysis has been performed by using computer software 'Graph pad prism'. $p<0.05\%$ is considered to be the level of significance. Statistical significance of frequencies of chromosome aberrations, micronuclei, erythrocytes Aberrations of exposed and control groups of each concentration and duration have been evaluated.
- For histopathology, specific target organs like gills, kidney and liver were taken as they were responsible for vital functions such as respiration, excretion and accumulation/biotransformation of xenobiotics in the fish. For present study gills, kidney and liver tissues were taken out after 120h for control as well as treated fishes of both the effluents and were fixed in Bouin's fixative. The fixed tissue was proceeded to study histopathological changes in tissues.
- Behavioural responses like erratic swimming, gulping of air, opercular movements, loss of equilibrium, hitting against the wall, restlessness and sluggishness, while morphological changes like loosening of scales, sinking of eyeball, redness in eye, profuse mucous secretions, bleeding from gills and haemorrhages in fishes of control and treated groups have been recorded. At 120h, in higher concentration two fishes died in

tannery effluent, while one fish died in paint effluent. The effect was more pronounced in tannery effluent as compared to paint effluent.

- Eleven types of chromosomal aberrations have been observed in the fishes. These are chromosome fragmentations, ring chromosomes, terminal chromatid deletions, minutes, centromeric gaps, terminal association of chromosomes, stickiness, clumping, pyknosis, stretching and pulverization. Chromosomal aberrations showed clastogenic effect in both the effluents. In tannery effluent at 1.93% concentration, there was decrease in all types of chromosomal aberrations with increase of exposure periods, particularly after 96h and 120h. Overall, the frequency of Cf was highest, followed by Tcd, Cg, whereas the frequency of Stch and Stk were lowest. The decreasing order of the aberrations was Cf>Tcd>Cg>Rc>M>P>Tac>C>Py>Stk>Stch. The mean frequencies of total aberrations were at 24h (45.66 ± 0.88^{a}), 48h (40.00 ± 0.57^{b}), 72h (35.00 ± 1.52^{c}), 96h (27.00 ± 1.73^{d}) and 120h (22.00 ± 0.57^{e}), which indicated that overall decrease in aberrations from 24h to 120h. However, in 7.74% concentration, there was increase in chromosomal aberrations from 24h to 120h. Overall, the frequency of Rc was highest, followed by Cf and Cg, while frequency of Stch, Stk and C were lowest. The decreasing order of the aberrations was Rc>Cf>Cg>M>Tcd>P>Py>Tac>C>Stk>Stch. The mean frequencies of total aberrations were at 24h (47.00 ± 1.52^{a}), 48h (53.66 ± 0.88^{b}), 72h (60.66 ± 0.33^{c}), 96h (67.33 ± 0.33^{d}) and 120h (72.66 ± 0.88^{e}), which showed the increasing trend of chromosomal aberrations from 24h to 120h.
- In paint at 3.95% concentration, there was decrease in chromosomal aberration from 24h to 120h. Overall, frequencies of M, Tcd, Cf were highest, whereas P and Stch were lowest and Stk, Stch and P were not observed at 120h. The decreasing order of the aberrations was M>Tcd>Cf>Rc>Tac>C>Cg>Stk> Py> Stch > P. The mean frequencies of total aberrations were at 24h (38.66 ± 0.88^{a}), 48h (31.33 ± 1.15^{b}), 72h

(26.00 ± 0.66^{c}), 96h (19.33 ± 1.72^{d}) and 120h (12.00 ± 1.45^{e}), which showed decreasing trend for aberrations from 24h to 120h. In 15.81% concentration, there was increase in chromosomal aberration from 24h to 120h. Overall, frequencies of Tcd, M, Cf were highest, while Stch and Tac were lowest and Stch was absent at 120h. The decreasing order of the aberration was Tcd>M>Cf>Rc>Cg>Py>C>P>Stk>Tac>Stch. The mean frequencies of total aberrations were at 24h (40.33 ± 0.66^{a}), 48h (46.00 ± 0.88^{b}), 72h (50.66 ± 1.15^{c}), 96h (57.00 ± 0.66^{d}) and 120h (68.33 ± 1.15^{e}), which indicated that increasing trend of aberrations from 24h to 120h. Mitotic indices also decreased in treated groups in both the effluents. The effect is more pronounced in tannery effluent as compared to paint effluent.

- Presence of one micronucleus is prominent as compared to two micronuclei and three micronuclei. In tannery effluent, at 1.93% concentration, mean frequency of micronuclei was decreased at 24h (26.33 ± 0.66^{a}) then steadily decreased at 48h (23.00 ± 1.15^{b}), at 72h (22.50 ± 0.57^{c}) and 96h (17.83 ± 0.33^{d}), while sharp decrease was detected at 120h (13.16 ± 0.88^{e}), whereas at 7.74% concentration, mean frequency of micronuclei was induced at 24h (32.16 ± 1.20^{a}) then steadily increased at 48h (37.66 ± 0.33^{b}), at 72h (42.33 ± 0.88^{c}) and at 96h (49.66 ± 1.45^{d}), whereas sharp increase was observed at 120h (56.66 ± 1.15^{e}). In paint effluent at 3.95% concentration, mean frequency was decreased steadily from 24h (15.66 ± 2.20^{a}), 48h (14.66 ± 0.66^{b}), 72h (12.33 ± 1.76^{c}), 96h (11.00 ± 2.30^{d}) and 120h (9.16 ± 1.45^{e}). However, 15.81% concentration, mean frequency was steadily increased from 24h (18.66 ± 0.88^{a}), 48h (23.33 ± 1.20^{b}), 72h (25.33 ± 0.66^{c}) and 96h (28.33 ± 2.02^{d}) and sharp increase was seen at 120h (35.83 ± 2.78^{e}). Frequency of micronuclei formation is more in tannery effluent as compared to paint effluent.
- Eleven types of erythrocytes Aberrations have been detected, which includes five nuclear aberrations (nuclear extrusion, blebbed nuclei, binucleated, lobed nuclei, notched nuclei) and six cellular aberrations

(enucleated cell, vacuolated cell, deformed cell, echinocytic, spindle shaped cell and apoptotic cell). In tannery, at 1.93% concentration, deformed, echinocytic and enucleated cells were predominant, while binucleated were lowest. The mean frequency of erythrocytes Aberrations were decreased from 24h (72.83 ± 1.20^{a}) to 48h (70.33 ± 1.76^{b}), 72h (63.83 ± 1.20^{c}) upto 96h (60.33 ± 1.76^{d}), while there was sharp decrease at 120h (46.50 ± 1.15^{e}). The overall decreasing trend for Aberrations was DC>EC>Enc>L>SC>VC>B>AC>NE>N>BN. In 7.74% concentration, deformed, spindle shaped and echinocytic cells were predominant, while binucleated were lowest. The mean frequency was increased from 24h (94.16 ± 2.08^{a}) to 48h (108.83 ± 1.20^{b}) then sharp increase was seen, after 72h (163.33 ± 0.66^{c}), 96h (177.50 ± 1.73^{d}) and 120h (195.83 ± 2.60^{e}). The overall decreasing trend was DC>SC>EC>VC>L>Enc>NE>N>AC>B>BN.

- In paint effluent, at 3.95% concentration, apoptotic, echinocytic and spindle shaped cells were predominant, while binucleated were very rarely seen. The mean frequency was decreased steadily from 24h (47.66 ± 1.20^{a}), 48h (40.66 ± 1.15^{b}), 72h (34.66 ± 2.60^{c}), 96h (29.66 ± 3.18^{d}) and sharp decrease was noted at 120h (19.33 ± 2.90^{e}). Overall, the decreasing trend for Aberrations was AC>EC>SC>NE>DC>N>L>Enc>VC>BN. In 15.81% concentration, echinocytic cell, nuclear extrusion and spindle cell were prominent, while binucleated were rare. The mean frequency was increased at 24h (69.16 ± 1.45^{a}), 48h (81.33 ± 0.66^{b}) and sharp increase was seen, after 72h (107.00 ± 2.30^{c}), 96h (137.00 ± 1.15^{d}) and 120h (154.66 ± 2.16^{e}). Overall, the decreasing trend for Aberrations was AC>EC>SC>NE>DC>N>L>B> Enc>VC> BN. Frequency of erythrocytes Aberrations is more in tannery effluent as compared to paint effluent
- The histopathology of gills shows haemorrhages, intracellular oedema, aneurysm, broken cartilage, damaged lamellae, loss of lamellae, lamellar

telangiectasia, necrosis, hypertrophy, hyperplasia, fusion of primary and secondary lamellae, curling of lamellae and lymphatic infiltrations, whereas kidney possesses haemorrhages, intracellular oedema, occlusion of tubular lumen, narrowing of tubular lumen, widen lumen, increased Bowman's space, shrunken glomerulus, decreased hematopoietic tissue, dilation of Bowman's capsule, necrosis, hypertrophy, hyperplasia and lymphatic infiltrations and liver reveals hemorrhages, aneurysm, intracellular oedema, disintegeration of central vein, fatty acid degradation, dilation of sinusoids, melano-macrophage centres, necrosis, hypertrophy, hyperplasia and lymphatic infiltrations.

- In tannery effluent, at 1.93% concentration, gills possess haemorrhages, intracellular oedema, lamellar telangiectasia, hyperplasia and lymphatic infiltration as significant alterations. On the other hand, in 7.74% concentration, gills damage is more severe than low concentration. Haemorrhage, intracellular oedema, broken cartilage, damaged lamellae, necrosis, hyperplasia, hypertrophy, lamellar telangiectasia, aneurysm and lymphatic infiltrations are significant changes. In paint effluent, at 3.95% concentration, damaged lamellae, loss of lamellae, lamellar telangiectasia and curling of lamellae are significant changes, while at 15.81% concentration marked changes are intracellular oedema, aneurysm, haemorrhage, damaged lamellae, loss of lamellae, necrosis, telangiectasia and lymphatic infiltration of cells.
- In tannery effluent, at 1.93% concentration, kidney shows presence of hypertrophy, hyperplasia and widen lumen of tubules as significant alterations, while, at 7.74% concentration, there is marked cellular and structural damage of the tissue, which includes mass intracellular oedema, haemorrhages, occlusion of tubules, necrosis, increased Bowman's space, shrunken glomerulus, dilation of Bowman's capsule, decrease in hematopoietic tissue, widen lumen, hyperplasia. However, in paint effluent, at 3.95% concentration, lymphatic infiltration, hypertrophy and dilation of Bowman's capsule are more pronounced

changes, whereas at 15.81% concentration, intracellular oedema, hypertrophy, lymphatic infiltrations, occlusion of tubular lumen, narrowing of tubular lumen, necrosis, shrunken glomeruls, increased Bowman's capsule, decreased hematopoietic tissue are marked alterations.

- In tannery effluent, at 1.93% concentration, liver reveals marked histological alterations as fatty acid degradation, dilation of sinusoids and presence of melano-macrophage centers, while, at 7.74% concentration, haemorrhage, disintegration of central vein, necrosis, dilation of sinusoids, melano-macrophage centres and fatty acid degradation are significant changes. In paint effluent, at 3.95% concentration, significant alterations are hypertrophy, disintegration of central vein, fatty acid degradation and dilation of sinusoids. On the other hand, at 15.48% concentration, melano-macrophage centers, hyperplasia, dilation of sinusoids, fatty acid degradation, intracellular oedema and aneurysm are significantly present.
- Histopathological alterations in gills, kidney and liver exposed to both the effluents indicate that severe damage occurred in tannery effluent as compared to paint effluent. The alterations are divided as circulatory changes, regressive changes, progressive changes and inflammation. In both the effluent mean index values of gills, kidney and liver are ranged in class II (index <10-25) at low concentrations, which represent moderate alterations, while at higher concentrations, these values are placed in class III (index <26-35), which show pronounced alterations in the organs with respect to control.
- I_{rp} index (Reactive index) values for both the effluents clearly depict that there are more regressive changes in the fishes treated with tannery effluent as compared to paint effluent. Moreover, the semi-quantitative results of both the effluents at low and high concentrations also indicate that regressive changes are more prevalent in all the selected organs (gills, kidney and liver). In both the effluents, there are more circulatory

disturbances than progressive changes at high concentrations, while progressive changes are more than circulatory disturbances at low concentrations. Inflammatory changes are present in the form of blood infiltrations but there are no tumor formations in all the tissues treated with effluents.

- Total reaction index ($TotI_{rp}$) values are 308 (7.74% concentration), 162 (1.93% concentrations) and 46 (control) of tannery effluent, whereas these values are 256 (15.81% concentration), 158 (3.95% concentration) and 32 (control) of paint effluent. In both the effluents, regressive changes are more prevalent. All these values represent the overall condition of target organs under the influence of various concentrations of both the effluents with respect to their controls.
- Organ indices (I_{org}) values are 364 (gills), 326 (kidney) and 292 (liver) for tannery effluent, while these are 324 (gills), 290 (kidney) and 254 (liver) for paint effluent. In both the effluent, gills are highly affected than kidney and liver because they come in contact with toxicants at first then by other tissues.
- Total organ indices ($TotI_{org}$) value is 982 for tannery effluent, whereas 868 for paint effluent, which indicate that organs are more damaged in tannery effluent than paint effluent.
- Fishes treated with two sub-lethal concentrations of tannery and paint industrial effluents for different time intervals resulted in chromosome aberrations, micronuclei, erythrocytes Aberrations and histopathology are showing concentration as well as time dependent responses. Higher concentrations of both the effluents proved to be more toxic than lower concentrations. However, lower concentrations cannot be ignored as they also induce genotoxicity and histopathological alterations which persist for long term effects.
- Thus, it is concluded that effluents discharge from industries cause genotoxic and tissue damage in fishes. The cumulative effect of both the effluents on fishes has been done for the first time, while few reports are

available on tannery effluent but effect of paint effluent has been done for the first time.

- ❖ Though, many laws regarding disposal of waste water has been given by our government but they are not followed, especially in case of small scale industries. It is highly recommended for both small scale as well as large scale industries to treat their waste water in treatment plant before dumping. Legal action should be taken to stop dumping of untreated wastes to rivers. Healthy aquatic environment should be maintained to save fish fauna as well as human health.