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Heavy Metal Induced Toxicity in Fish with Special Reference to Zinc and Cadmium

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Abstract
Heavy metals are commonly found in water pollutants these days. Toxicity as a result of heavy metals may adversely affect the aquatic flora and fauna. Growth parameters, morphological characteristics and haemotological, biochemical and physiochemical parameters of fish may serve as important bio-indicators against this type of pollution. This in turn may help in timely proper management of water pollution as well as saving the aquatic life from damage, biomagnification, disturbance in food chain and extinction.

Keywords: Heavy metals, toxicity, fish, enzymes, growth parameters.

Introduction
Metals are unique among pollutants, in that they occur naturally and in many instances are ubiquitous in the environment, but may cause adverse health effects as well. Natural resources serve as habitat for various aquatic fauna and flora. It is generally believed that every water body is capable of accepting certain minimum amount of pollution without any adverse effect on itself due to natural biological cycles and self-purification capacity. Pollution of water with heavy metals may adversely affect the immune system of fish leading to decreased production, increased susceptibility to diseases and mortality. Cadmium (Cd) metal has a high profile in human toxicology where it has been transferred at high concentration through food chain. In water, the main point source is effluents from electroplating works. Cd is strongly adsorbed as organic and inorganic particles in water. Although it can form soluble complex with humic substance, but the toxicity is not reduced like that in case of Cu [1]. Zinc (Zn) metal is widely detected in freshwater although Zn compounds have relatively high solubility. Zn is an essential element for aquatic life. For example, Zn occurs in the enzyme carbonic anhydrate and catalyses the formation of carbonic acid from carbon dioxide in the blood. Therefore, small amount of Zn in the water or in the diet is essential. The organisms will have internal mechanisms to transport Zn around the body in order to manufacture such vital enzymes. When the Zn in water rises to a level, the amount entering the organism through the gills exceeds the requirement for this metal. It was originally thought that the direct toxic action of Zn on fish was to precipitate the layer of mucus on the surface of the gill, causing suffocation [2, 3].

Many workers have studied that haemotological parameters of fish are used as indicator of their physiological state and their study has become widespread in the control of pathogens and manipulation of stress in fish. Literature survey shows the influence of many factors like heavy metals [4] pesticides [5] and age, stress due to industrial waste, handling [6] etc. Yoshitomi *et al.* [7] concluded that Cd contamination had morphological effect in the form of scale deformation in common carp, *Cyprinus carpio.* Sesha Srinivas and Rao [8] study of effect of 96 h LC_{50} concentration of hexavalent chromium (39.40 mg/l) on the gills of *Labeo rohita* and revealed damage, fusion, bulging at distal parts and necrosis of secondary gill lamellae and atrophy of central axis. Rani [9] observed congestion of primary rachis, bulging of primary and secondary gill lamellae, hypertrophy and hyperplasia of interlamellar cells, atrophy of several of secondary gill lamellae and necrosis in the gills of *Oreochromis mossambicus* with reference to Cd toxicity. Toxicity induced damage to gill surface is further corroborated from various other studied. Hypertrophy and thinning of epithelial cells in gill structure due to reaction to the heavy metals were adaptive responses to enhance oxygen diffusion during

stress, whereas secretion of mucous was to prevent the entry of toxicant through gill surface. Farkas *et al.* [10] studied that carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood. The toxic effects of heavy metals have been reviewed, including bioaccumulation. The organisms developed a protective defense against the deleterious effects of essential and non-essential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body. Shah [11] observed behavioral abnormalities of fish *Cyprinion watsoni* on exposure to different concentrations of Cu and Zn for a week. A concentration of 0.06 mgCu/l caused increased swimming activity and breathing movements. The highest treatment of Cu (0.12 mgCu/l) caused lethargy and loss of equilibrium in fish. Stress responses and changes in protein metabolism were studied by De-Smet and Blust [12] in common carp, *C. carpio* exposed to 0, 0.8, 4 and 20 µM Cd over a 29 day period. Cd accumulated in the tissues in the following order: kidney>liver>gills. The concentrations of Cd and Zn binding metallothioneins were in the order: liver>kidney>gills. The activities of proteases were increased at day 4 in gills, liver and kidney of carp.

The relationships among growth, feeding behavior, ion regulation, swimming performance and oxygen consumption in rainbow trout (*Oncorhynchus mykiss*) were compared by McGeer *et al*. [13] during chronic exposure to sub-lethal levels of water born Cd, Cu and Zn. Feeding but appetite was increased and decreased in Cu and Cd exposed trout respectively. Critical swimming speed was significantly lowered in fish chronically exposed to Cu. Sharma [14] studied the effect of 96 h LC_{50} concentration of hexavalent chromium on the gills of *L. rohita* and revealed damage, fusion, bulging at the distal parts, the necrosis of the secondary gill lamellae and atrophy of the central axis. It also reported ruptured tips of villi and wide intercellular spaces between them when *C. mrigala was* exposed to the heavy metals. Poleo *et. al*. [15] found that aluminum (Al, 50-200 mg/l) and Zn (Zn, 50-400 mg/l) impaired *Gyrodactylus turnbulli* population growth. Cu (10-80 mg/l), iron (25-250 mg/l) and manganese (100-800 mg/l) did not affect these parasites. Recently, Gheorghiu *et al.* [16] reported a concentration dependent increase in mortality of guppies in response to the combined effects of aqueous Zn and infection by *Gyrodactylus turnbulli.* Joshi and Kulkarni [17] found the LC_{50} values for 24, 48, 72 and 96 hrs, 0.179, 0.147, 0.116 and 0.0787ppm respectively for cypermethrin, and 0.258, 0.218, 0.177 and 0.147ppm respectively for fenvalerate. Erratic swimming, difficulty in respiration, jerky body movement, rapid opercular movements prior to death were observed in the fish under toxic stress. Cypermethrin was found to be more toxic than the fenvalerate in *Garra mullya*. Agzhari *et al.* [18] found the activity of a few biomarkers on the freshwater fish *Channa punctatus* treated with monocrotophos for acute exposure to 18.56 ppm at 96 hrs and subacute exposure viz., 0.46 ppm, 0.96 ppm and 1.86 ppm for 30 days. The protein levels were found to be depleted in all the tissues after the lethal and sub-lethal concentration of the pesticide exposure as compared to the control whereas the lipid levels showed an increase under the stress of pesticide monocrotophos. C. *punctatus* were exposed to three sub-lethal concentrations of Zn (10 mg/l, 15 mg/l and 25 mg/l) for 15 days. The effects of this exposure have been studied on bioaccumulation of Zn and histology of the kidney at an interval of 8, 10 and 15 days. Statistically, a significant increase in the Zn concentration was noted in fish of all the treated groups. Simultaneously, several histological changes were also noted in the kidney of all the exposed groups. According to Taylor *et al.* [20] chronic exposure of fish to water-borne Cu, Cd or Zn has been shown to cause a variety of physiological and behavioral changes including loss of appetite, reduced growth, ionic loss and increased fish mortality. However, these effects were not consistent and some studied have shown that there could be minimal physiological disturbances even when exposure to Cd, Cu and Zn were sufficient to cause some mortality in fish. Abel and Papoutsoglou [21] investigated the sub-lethal toxicity and accumulation of Cd in *Tilapia aurea.* The Cd appeared to have no effect on the growth or on the moisture, fat protein or ash content on hemoglobin concentration of fish. Hematocrit values of fish examined after ten weeks exposure showed a general decline with increasing Cd concentration. Cd delayed growth hormone (GH) expression during rainbow trout development. They demonstrated *in vivo* endocrine disrupting capacity at the molecular level for Cd in teleosts.

Lin and Hwang [22] observed that many metal toxicity with both juveniles and adult fish but due to their sensitivity to environmental toxicants, fish embryo and larvae were frequently favored as bio-indicator for water quality. Bio-assay allowed the detection of this effect by measuring the biological response of aquatic organisms, particularly in their highly sensitive early life stages. Especially, toxicity test with early stages supplied more information on mortality and numerous other significant endpoints such as malformations, growth inhibition and developmental process delays. The Cd was found to be acutely toxic to larvae of the test fish at high concentration ranging from 750 to 3000 µg L^{-1}. The calculated LC_{50} value was 1921 µg Cd L^{-1} for 96 h and 382 µg Cd L^{-1} for 21 days of exposure. The mortality response of *H. fossilis* larvae during chronic exposure was found to be 5 times more sensitive than those exposed to acute toxicity (96 h) of Cd. Exposure duration evidently affected sensitivity of fish larvae and influenced the value of LC_{50}. The greater sensitivity of larvae to Cd toxicity was also observed in test with *Salmo gairdneri.*

Ali *et al.* [23] reported reduced growth rate of rockfish (*Sebartes schlegeli*) due to Cu stress and there was an inverse relationship between growth and Cu exposure and reported that the feeding parameters were greater in control fish that decreased with increasing concentration of Cu in ornamental fish. Goldoni *et al.* [24] indicated that although trace metals were essential for normal physiological processes, abnormally high concentrations were toxic to aquatic organisms. Living organisms required varying amounts of some heavy metals for their metabolic function; for instance, chromium played an important role in growth and metabolism (carbohydrates, glucose, cholesterol, and lipids) but at higher concentrations (200-300 mg/L for freshwater fish), chromium was a highly toxic, mutagenic [25], carcinogenic [26], teratogenic, highly mobile and incorporating metal in the food chain.

Javed *et al.* [27] reported that extra pure Cu chloride ($CuCl_2$. $6H_2O$) was used to prepare stock solution of required metal dilution. One third of Cu (Cu) LC_{50} concentration of 19.44 mg L^{-1} was used as sub-lethal level for *Catla catla*. The treated fish were kept in the aquaria containing sub-lethal concentration of water-borne Cu and grown for 60 days, while control fish were placed in metal free water. Physico-chemical parameters viz. temperature, pH, total hardness, dissolved O_2, total NH_3, Na, K and CO_2 of the treated and control media

were monitored on daily basis by following the methods of APHA [28]. The maximum increment in the average weight of fish was 1.16 g during the 8th week, followed by that of 0.43 g in the 6th week while this fish showed decrease in its weight during 1st week of this study period. It was observed that chronic exposure to the fish exerted significant impact on fish growth as control fish showed significant wet weight, fork and total length gains than that of Cu treated fish. Chronic water-borne Cu stress on the fish exerted a significant ($p<0.001$) impact on its growth, determined in terms of average wet weight, fork and total length increments. The wet weights were significantly variable between treated and control fish.

Sehgal and Saxena [29] studied uptake of Zn in few tissues of fish, *Puntius sophare,* exposed to 7.5 mg/l of $ZnCl_2$ for 48 and 96 hrs. Intestine accumulated the highest amount of Zn on dry weight basis followed by gills, liver and kidney. Kaviraj and Konar [30] determined the LC_{50} and LC_{95} values of Hg-Cr-Cd mixture for the fish *Tilapia mossambicus* which was in the range of 90.0 to 190.5 ppm. The acute toxicity of Cd and Zn to *Puntius ticto* and reported the LC_{50} for 24, 48, 72 and 96 h as 35, 30, 28 and 26 ppm for Cd and 75, 70, 66 and 60 ppm for Zn respectively. Gill *et al.* [31] exposed rosy barb (*Puntius conchonius*) to 181 µg/l mercuric chloride for 48 h and the activity of acid and alkaline phosphatases (AcP and AIP), aspartate aminotransferase (AAT), alanine aminotransferase (AIAT), lactic dehydrogenase (LDH), and acetylcholinesterase (AchE) were measured *in vivo* in several organs. An increase in LDH activity occurred in the cardiac and skeletal muscles while the AchE activity was considerably lowered in the brain, gills and liver. Sastry and Shukla [32] reported decrease in the activity of glucose-d-phosphatase and increase in the activities of hexokinase, lactate dehydrogenase and succinate dehydrogenase in liver and muscles of *Channa punctatus* chronically exposed to Cd for 15 and 30 days. The enzyme activities decreased after 60 days of exposure. Radhakrishnaiah *et al.* [33] exposed freshwater fish *Cyprinus carpio* to the sub-lethal concentration of mercury (0.1 mg/l) and Zn (6.0 mg/l) which resulted in distinct changes in the energy metabolism of gill, liver and muscle at 1, 15 and 30 days. The activity of LDH and the levels of pyruvate and lactate increased in all the three organs of the fish at the three exposure periods studied in both the metal media. But, this increase was also in the order 1 > 15 < 30 days and 1 > 15 > 30 days in the organs of the fish exposed to mercury and Zn, respectively.

The effects of sub-lethal concentrations of mercury (0.1 mg/l) and Zn (6 mg/l) on acetyl cholinesterase activity and acetylcholine content of gill, kidney, intestine, brain, liver and muscle of the freshwater fish *Cyprinus carpio* at 1, 15 and 30 days of exposure were studied by Suresh *et al.* [34]. A significant suppression in acetyl cholinesterase activity was recorded in all the organs from both mercury and Zn intoxicated fish at all the exposure periods. Sastry and Shukla [35] studied decreased activities of glucose-6-phosphatase, hexokinase, lactate dehydrogenase, succinate dehydrogenase and malate dehydrogenase in liver and muscles of *Channa punctatus* after acute and chronic exposures to Cd (1.12 mg/l) for 120 days. Shakoori *et al.* [36] reported that Lactate dehydrogenase (LDH) is an oxidoreductase, which catalyzes the inter-conversion of lactate and pyruvate depending on the availability of NAD (Coenzyme). The decrease in LDH activity suggests a reduction in the conversion of lactate to pyruvate, thereby leading to the accumulation of lactic acid. The decrease in LDH activity with a consequent increase in the levels of lactic acid suggests the predominance of anaerobic segment, glycolysis. The change in the level of activity of this enzyme was extensively used as an indicator of stress in fish. However, decrease in LDH activity in different organs due to pesticide influence. Sharma *et al*. [14] estimated bioaccumulation of Zn and Cd in freshwater fish's *viz*. *Labeo rohita, Catla catla, Channa punctatus* and *Poecilia reticulata* after acute and sub-lethal exposure. The accumulation behavior of Cu, Zn, Cd and Pb concentrations in flesh, gills, liver and gonads of the commercial fish *Mullus barbatus, Merluccius merliccius and Boops boops* from the Aegean sea in Turkey have been studied by Zyadah and Chouikhi, [37]. Santhana and Azariah [38] noticed disturbance in enzyme activities of the Crab *Sesarma quadratum* after exposing to two sub-lethal concentrations of Cu chloride for 21 days. Lactate dehydrogenase (LDH) activity was significantly elevated in muscle and hepatopancreas tissues whereas succinate dehydrogenase (SDH) activity was suppressed in the crab tissues of the muscle, gills and hepatopancreas for 21 days. Dinodia *et al*. [39] reported that maximum decline in proteolytic enzyme activity was reported in fish *Cirrhinus mrigala* after an exposure to Cd at 5.0 ppm. The effects of heavy metals (Cd^{2+}, Cu^{2+}, Pb^{2+} and Zn^{2+}) on activities of carp trypsin, alpha-chymotrypsin, carboxypeptidase A and lipase were studied by Kotorman *et al*. [40]. Presence of Cd^{2+} inhibhited alpha-chymotrypsin and lipase activity 10-20%. Cu^{2+} only slightly influenced the trypsin and lipase activities, whereas the alpha-chymotrypsin activity was decreased. Lionetto *et al*. [41] studied *in vitro* effect of Cd on enzymes such as intestinal and branchial carbonic anhydrase and Na^+K^+ATPase. Carbonic anhydrase were significantly inhibited by $CdCl_2$, the gill carbonic anhydrase was more sensitive to the heavy metal. With the exception of trypsin, Pb^{2+} inhibited the activities of all investigated enzymes.

Martinez *et al.* [42] investigated lead effects on gill morphology, haematocrite, blood sodium, glucose, lipid, protein and cholesterol of *Prochilodus lineatus* exposed to two sub-lethal lead concentrations for 96h. Gills of *P. Lineatus* exposed to both lead concentrations during 96h presented a higher occurrence of histopathological Lesions. Kothari and Soni [43] reported sub-lethal exposure of $ZnSO_4$ (150 mg/l) to fresh water cat fish *H. fossillis* for 30 days, caused significant alteration in gill function as revealed by elevated alkaline phosphates over control. Simultaneous feeding of Poly unsaturated fatty acids to fish maintained these parameters near normal values. Nevalennyi and Bednyakov [44] reported the influence of Cd ions at 0.25 mg/l on the alpha-amylase, maltase and alkaline phosphatase activities of carp, *Cyprinus carpio*. Alkaline phosphatase activity remain unchanged after 10 days then increased to peak on day 30 and decreased to 40-50% of control at 50-60 days. Patil and Hande [45] conducted a study on toxic effect of Zn chloride on brain acetylcholinesterase of a marine teleost, *Arius nenga.* It was observed the Zn exerted the inhibitory effect on cytoplasmic and membrane bound fractions of AchE. Van Aardt and Booysen (2004) observed effects of Cd on oxygen consumption, plasma chloride and bio accumulation in *Tilapia sparrmanii.* Das *et al*. [46] studied the effects of nitrate toxicity on hematological parameters of fingerlings of Rohu *(Labeo rohita).* In the experiment, fingerlings *L. rohita* were exposed to 0, 1, 2, 4, 8 and 10.4 mg/l nitrite for 96 hours. Blood parameters of *C. carpio* exposed to sub-lethal concentrations of Cd, nitrate and mercuric chloride (0.30 ppm) for 90 hours. The results showed that there was significant decrease in

erythrocyte count, hematocrit and hemoglobin content. However, the leucocyte count, thrombocyte count and blood clotting time of the fish did not change significantly. Satyaparameshwar *et al.* [47] observed the sub-lethal toxicity of Cu sulphate on carbohydrate metabolism in selected tissues of freshwater mussel, *Lamellidens marginalis*. The levels of glycogen and pyruvic acid decreased while lactic acid showed an increase. Activities of LDH, SDH and MDH decreased while G-6-PDH activity increased. There appeared to be a shift in the carbohydrate metabolism from aerobic to anaerobic type due to toxicity of Cu.

In vitro effects of individual heavy metal ions as well as their combinations on catalase activity of *Sarotherodon mossambicus* were studied [48] Cu was found to be the strongest inhibitor of catalase activity followed by mercury, iron, and chromium and Cd. Cu toxicity on catalase activity was reduced in the presence of all the other metal ions. However, it has been observed that the addition of Cd, chromium, iron, manganese, lead, mercury, nickel, lead, and Zn increased their inhibitory effects on catalase activity. The effect of heavy metal (Cd^{2+}, CU^{2+}, Pb^{2+} and Zn^{2+}) on activities of carp (*Cyprinus carpio*) for the trypsin, alpha-chemotrypsin, carboxypeptidase A and lipase were studied. Begum [49] studied alterations induced by cypermethrin in the fish, *Clarias batrachus*. The fishes were exposed to cypermethrin at a concentration of 0.07mg/l for 10 days. After 10 days, fish were released into fresh water to observe the recovery response. At the end of 1, 5 and 10 days of exposure, reduction in protein was observed in both tissues and recovery response was seen in muscle and kidney. It was also found that transaminases (ALAT and AAT) and glutamate dehydrogenase (GDH) activities were increased in both tissues for 10 days exposure span. Vinodhini and Narayanan [50] determined the bioaccumulation of heavy metals in various organs of the fresh water fish *Cyprinus carpio* exposed to Cr, Ni, Cd and Pb at sub-lethal concentrations for a period of 32 days. The accumulation of heavy metal gradually increased in liver during the heavy metal exposure period. The order of heavy metal accumulation in the gills and liver was Cd > Pb > Ni >Cr and Pb > Cd > Ni > Cr. Similarly, in case of kidney and flesh tissues, the order was Pb > Cd > Cr > Ni and Pb > Cr> Cd > Ni. In all heavy metals, the bioaccumulation of lead and Cd proportion was significantly increased in the tissues of the fish. Firat and Kargin [51] found that Cr (VI) exposure caused significant elevation in transaminases (serum glutamic oxaloacetic transaminase and serum glutamic pyruvic transaminase), acid phosphatase (ACP), and alkaline phosphatase (ALP). Blood cell changes associated with Cr (VI) included decreased erythrocyte count and hematological indices. Other changes associated with Cr(VI) exposure included hypercholesterolemia and hypoglycemia; changes in blood cell indices were variable depending on the concentration and period of exposure [26, 52, 53]

Hilmy *et al*. [54] studied changes in total protein liver, heart, gill and serum enzymes after exposing fish *Mugil cephalus* or tissue homogenates and serum to Cd. Total protein content in the exposed fish showed an increase in the liver, gill and serum, while there was no change in heart protein. The sensitivity of the assayed enzymes to Cd varied in different tissue. James *et al.* [55] reported toxic effects of Cu, Zn and Cd in *Oreochromis mossombicus.* Cu was most toxic followed by Zn and Cd. Combination of Zn+Cd was less toxic than the combination of Cu+Cd and Cu+Zn. A significant decrease in protein, carbohydrate and lipid were observed in muscles, liver, gills and the whole body of *O. mossambicus* exposed to metals individually and in combinations except when exposed to Cu alone. Combinations of Cu+Cd, Cu+Zn and Zn+Cd markedly reduced the protein, carbohydrate and lipid in all the tissues tested. Brafield and Koodie [56] analysed the effect of high dietary Zn on trypsin activity in carp, *C. carpio*. Carp fed with pellets containing a range of Zn concentration showed a significant increase in trypsin activity in the intestine with increasing Zn in diet. Golovanova *et al*. [57] studied *in vitro* effects of Cd (0.5 – 50 mg/l) and DDVP (dichlorovos, at 0.2-100 mg/l) on the total amylolytic, sucrose and protease activity of intestinal mucosa for the first time in eleven fresh water teleosts. Total amylolytic activity in burbot, *Lota lota*, crucian carp, *Carassius auratus,* and common carp, *Cyprinus carpio*, sucrase activity in burbot and pipe were significantly decreased by Cd at 50 mg/l, DDVP (at 0.2 mg/l) caused a significant decrease in total proteolytic activity in pipe, had no effect on either protease or carbohydrase activity in other fish species.

Hidalgo *et al.* [58] conducted a comparative study on the digestive and amylolytic activities in six species of fish with different nutrition habits. Trout and carp showed the highest digestive proteolytic activity. Eels showed the lowest digestive proteolytic potential among the species studied. The ratio of total amylase to total proteolytic activity was higher in omnivorous fish species. Rema and Babu [59] studied the effect of Zn and mercury on the activities of the enzymes, acid phosphatase, aspartate aminotransferase and glutamate dehydrogenase in *Oreochromis mossambicus* and reported a variability in activities of enzymes depending upon the duration of exposure to the toxicant. Dinodia *et al*. [39] investigated the effects of Cd toxicity on proteolytic enzyme activity in fresh water carps and it was reported that proteolytic enzyme activity declined maximally in fish *C. mrigala* from 26% at 2.5 ppm to 55.60% at 5.0 ppm. Virk and Sharma [60] assessed the effects of acute toxicity of nickel and chromium on fingerlings of the *C. mrigala.* After 45 days of exposure, significant decline in the protein and carbohydrate content of gills was observed. Bhatkar *et al.* [61] determined sub-lethal effects of $CrCl_2$, $NiCl_2$ and $ZnCl_2$ on the biochemical parameters of the fish *L. rohita* for 30 days. After 10 days of exposure to $CrCl_2$, $NiCl_2$ and $ZnCl_2$, blood glucose level increased by 48.11%, 71.71% and 68.35% respectively. However, after 20 and 30 days of treatment, significant decrease in glucose level was recorded. Protein and glycogen content of liver and muscle were depleted in all the experimental fish. Hameed and Muthu [62] studied two sub-lethal concentrations (10% and 30% of LC 50) of Cd for a period of 96 hrs on the fish *Oreochromis mossambicus*. The carbohydrate, protein and the lipid content of fish decreased with time due to increased Cd concentrations. The effects of Cd toxicity on the biochemical, enzymatic and hematological parameters in the fresh water fish species *L. rohita, C. mrigala* and *C. carpio*. A maximum decline in the carbohydrate content was reported to be 19.2, 42.46 and 38.3 percent in *L. rohita, C mrigala* and *C. carpio* respectively. A decline in the protein content was 24.3 to 25.5 percent and that in the liver glycogen was from 11.2 to 45.2 percent. The proteolytic enzyme activity declined maximally (32.6%) in *L. rohita* due to Cd toxicity.

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