Original Research Article

Effects of Chromium (VI) on haematological parameters ~~in freshwater~~ ~~food~~of~~fish~~, *Channapunctatus*(Bloch. 1793)

Abstract

Numerous enterprises discharge their untreated waste water straight into aquatic habitats. In addition to causing water pollution, the poisonous heavy metals like chromium found in that waste water also have an impact on aquatic life. Therefore, the goal of this research was to assess the toxicological effects of chromium (VI) on *Channapunctatus*, a freshwater fish. Chromium is an important metal and also a severe threat to aquatic life. Fish, the dominant creature in the aquatic environment, end up being the main target of these contaminants. Chromium enters fish's bodies via their gills, skin, and digestive tracts as a result of contaminated food. They are eventually carried by the circulation to the organs and tissues, where they accumulate after absorption. Through the food chain, heavy metals ultimately find their way into people. Fish were subjected to three different amounts of chromium ($LC_{50}/5$: 15.37 mg/L, $LC_{50}/10$: 7.68 mg/L, and $LC_{50}/20$: 3.84 mg/L) in this experiment, as well as a toxicant-free control. The relevant blood samples were taken from the caudal peduncle at intervals of 7, 14, 21, and 28 days in order to determine haematological parameters (haemoglobin, haematocrit, red blood cell count, etc.). As a consequence, several alterations in these parameters were noted, and eventually, fish began to lose their resistance to disease, become anaemic, and weaken their immune systems. While it was stated that throughout exposure periods of 7, 14, 21, and 28 days, Groups 2, 3, and 4 had substantially higher MCH and MCV values than Control (Group 1), it was also observed that Groups 2, 3, and 4 had lower Hb~~%~~, RBC, WBC, and PCV~~%~~ values. The primary objective of this research was to increase public awareness of the need to protect ~~preserve~~ aquatic environments, particularly from ~~those that are~~ heav~~il~~y metal-contaminationed.

Comment [A1]: How many fishes?

Comment [A2]: Other parameters also have their units. This should be reflected in the table.

Key words: Haematological parameters, haemoglobin, *Channapunctatus*, chromium trioxide.

Introduction

Because heavy metals are persistent and non-biodegradable, and because they tend to accumulate in living things, environmentalists are now highly worried about water pollution brought on by heavy metal contamination (Javed et al., 2016). Heavy metals in the atmosphere are irreversible, so they either accumulate in biota or leach into groundwater (Islam et al., 2018). Heavy metals are essential to many biological processes in aquatic species and are still present in the body in trace amounts, or in amounts that do not surpass 1 μg/g. However, even a little concentration rise causes substantial levels of toxicity in several organs.One of the most typical pollutants in surface and ground water is chromium (Cr). In an aquatic ecosystem, Cr travels through effluents that are primarily released from different sources, including metal finishing, dyeing, and printing industries, leather tanneries, mining, photographic, pharmaceutical, electroplating, ceramic, and textile industries, etc. (GracePavithra et al., 2019; Liknaw et al., 2017; Ukhurebor et al., 2021). On the other hand, insufficient treatment of these effluents might lead to Cr (VI) contamination of neighbouring water bodies, where it is often found at concentrations that could be harmful to fish (Bhatia, 2017; Lellis et al., 2019). Cr (VI) interacts negatively with the environment due to its capacity to maintain persistence and produce a broad variety of unfavourable effects on biological and ecological systems, including fish and aquatic life (Ali et al., 2019).Consuming chromium-tainted aquatic food has raised the danger of human health issues worldwide, but especially in developing nations like India. Fish are thus regarded as one of the most crucial indicators in aquatic systems for tracking trace metal contamination and its potential effects on human health (Javed et al., 2016). Since fish make up the majority of the aquatic creatures in this system, they are also the most susceptible to these poisons. Chromium is ingested by fish either via their gills or their stomachs, where it circulates throughout the body before being subsequently deposited in different organs (Al-Taee et al., 2020). Heavy metal contamination reportedly reduced fish production (Lu et al., 2015).

In many fish species, haematology serves as a gauge of overall health. Fish blood's haematological indicators may tell us a lot about how physiologically a fish reacts to changes in its environment. For many years, metal pollution in the aquatic environment has been monitored using fish haematological indicators (Authman, 2015). Blood indices are thus considered pathophysiological markers of the whole organism in order to assess whether fish have suffered structural and functional damage as a consequence of toxic exposure (Fazio, 2019). Fish exposed to heavy metals may have changes in their blood parameters. Diverse impacts on the levels of Hb and Hct have diverse effects on the blood's capacity to transport oxygen depending on the kind of heavy metal, the species, and the time of exposure. Numerous hematological indicators, including haemoglobin (Hb), haemoglobin concentration (Hct), red blood cells (RBCs), and others, have been employed in aquatic settings as indicators of metal pollution and are routinely used to assess the functional status of the bloodstream's capacity to supply oxygen (Fazio, 2019). The author chose to focus on chromium among all the heavy metals since there are several tanneries close to the Lucknow region that ultimately dump chromium.Investigating the effects of chromium toxicity on several *Channapunctatus*’s~~H~~haematological parameters is the main goal of the current investigation. Since environmental toxicants primarily affect fish among aquatic organisms and, as a result of these pollutants, fish health deteriorates over time, an attempt has been made to correlate the impact of these pollutants on the fish's initial health markers, namely, selected ~~H~~haematological parameters, in the current experiment.

Materials and Methods

Test Animal

Channapunctatus, a freshwater edible fish, were collected from local lentic habitat of Lucknow with the help of a local fisherman. The fish were brought to the Lucknow University Laboratory of Zoology Department in a plastic container with water inside that was covered with a cotton rag to keep the fish from escaping. Fish were ~~incubated~~immersed in a 0.1% KMnO4 solution for three to five minutes to remove any contaminants from the dermal region. The fish were accustomed to laboratory conditions for 15 days in aquariums with 100 L of fresh tap water prior to the experiment. During the acclimatisation period, fish were fed optimal fish food twice daily. Every aquarium was regularly examined for fish mortality, and those that were found dead were removed from the tanks.

Test Chemical

The test chemical was made by Merck Specialities Private Limited, Shiv Sagar Estate Worli, Mumbai, 400018), and was purified CrO_3 Chromium (VI) Oxide. ~~Other~~ All compounds that were employed in different formulations were of the highest commercial grade.

Comment [A3]: The other half of the bracket is missing.

Experimental approach

There were two sets used to conduct the experiment. The EPA Probit Analysis Programme was used to calculate the LC_{50} values for CrO_3 during the course of the first set of experiments, which lasted 96 hours. The fish were split into four groups of 30 fish each for the second set. While fish from groups 1 retained as the control group with no treatment, fish from groups 2, 3, and 4 were given sub-lethal doses of chromium trioxide for 28 days at LC_{50}/20: 3.84 mg/L, LC_{50}/10: 7.68 mg/L, and LC_{50}/5: 15.37 mg/L, respectively. The experimental set up received appropriate aeration. After the study started, mortality was tracked every day, and dead fish were immediately removed.

Haematological Analysis

After 7, 14, 21, and 28 days respectively, blood samples from the fish that had survived in each tank were obtained using the caudal vein technique for ~~a~~ haematological study. The Neubauerhemocytometer was used to count the RBCs and WBCs under a light microscope. Haemoglobin (Hb%) was determined using haemoglobin strips and a digital EasyMate® GHb (Model: ET 232, Hb/Glu double monitoring device, Bioptic Technology Inc., Taiwan 35057). According to Jain (1993), the packed cell volume (PCV, %), mean corpuscular volume (MCV, m3), and mean corpuscular haemoglobin (MCH, pg) were all calculated using the formulae below.

PCV (%) = % Hb x 3

MCV (μm^3) = (PCV/RBC in millions) x 10

MCH (pg) = (% Hb/RBC in millions) x 10

Evaluation of the data and statistical analysis

The standard deviation (SD) was used to represent values. By using probit analysis, the LC_{50} 96 hrs value of chromium was calculated. A one-way ANOVA was used to analyse the data, and then a post hoc test was used to determine if there were any statistically significant differences between treatment and control values. At a significance level of $p< 0.05$, statistical analysis was carried out using SPSS version 27.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Comment [A4]: Test of significant not mentioned in the results. Author should state whether significant difference were observed.

The freshwater fish *Channapunctatus*' ~~H~~haematological parameters for 7, 14, 21, and 28 days were studied in the present research to determine the impact of sub-lethal amounts of chromium trioxide on them. RBC count, Hb%, PCV, MCH, MCV, and WBC count were among the variable Hematological indicators. After being exposed for 28 days to various concentrations of chromium trioxide, the fish in group 1 (the control) had haemoglobin percentages that ranged from11.00 ± 0.50 to 11.20 ± 0.20; however, these values dropped to 10.20 ± 0.20 to 10.80 ± 0.50 in group 2, 9.60 ± 0.20 to 10.30 ± 0.50 in group 3, and 8.20 ± 0.20 to 9.50 ± 0.30 in group 4. Fish in group 4 had the greatest Hb% decline. When compared to control, group 4's RBC count value fell the most. The RBC count ranged from 2.21 ± 0.21 to 2.23 ± 0.36 in group 1, whereas it ranged from 1.00 ± 0.32 to 1.09 ± 0.32 in group 4. In comparison to group 1, groups 2 and 3 similarly showed a drop in RBC count. The packed cell volume (PCV)% values for groups 2, 3, and 4 were significantly lower than those for group 1, with group 1 having the highest values, which ranged from30 ± 1.00 to 30.33 ± 1.60, and group 4 having the lowest values, which ranged from 25.00 ± 0.50 to 25.50 ± 1.50. The white blood cell count was substantially lower in groups 2, 3, and 4 than in group 1, with group 4 having the lowest number (560) and group 1 having the highest (3,166). On the other hand, after a 28-day exposure to chromium trioxide, mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) levels were significantly higher than controls. MCH values for groups 2, 3, and 4 ranged from 50.45 ± 4.15 to 54.33 ± 4.15, 65 ± 3.26 to 70.15 ± 1.20 and 70.23 ± 3.88 to 77.55 ± 3.44, respectively. Group 1 had the lowest MCH values, which ranged from33.48 ± 4.68 to 35.48 ± 4.50. MCV values in group 4 ranged from 202.33±5.78 to 239.45±3.57, whereas those in group 1 ranged from 82.14 ± 4.26 to 85.23 ± 4.12. Group 4 had the highest MCV values. Values for groups 2 and 3 were likewise raised in comparison to group 1.

Comment [A5]: Insert the unit before the semi colon.

Parameters	**Exposure time (days)**	**Group1 (control) Mean ±SEM**	**Group 2 (LC_{50}/20) Mean ±SEM**	**Group 3 (LC_{50}/10) Mean ±SEM**	**Group 4 (LC_{50}/5) Mean ±SEM**
RBC Count (millions/cumm)	7	2.21 ± 0.21	2.15 ± 0.48	1.65 ± 0.43	1.09 ± 0.32
	14	2.21 ± 0.52	2.15 ± 0.55	1.58 ± 0.37	1.06 ± 0.32
	21	2.23 ± 0.36	2.04 ± 0.33	1.40 ± 0.33	1.02 ± 0.43
	28	2.22 ± 0.34	2.00 ± 0.26	1.40 ± 0.32	1.00 ± 0.32
Haemoglobin (Hb) (%)	7	11.20 ± 0.20	10.80 ± 0.50	10.30 ± 0.50	9.50 ± 0.30
	14	11.13 ± 0.30	10.60 ± 0.60	10.30 ± 1.20	9.20 ± 0.30
	21	11.10 ± 0.50	10.50 ± 0.30	10.10 ± 0.50	8.60 ± 0.60
	28	11.00 ± 0.50	10.20 ± 0.20	9.60 ± 0.20	8.20 ± 0.20
Hematocrit (PCV %)	7	30.33 ± 2.50	29.83 ± 1.50	27.50 ± 1.15	25.50 ± 1.50
	14	30.33 ± 1.60	29.50 ± 1.30	27.30 ± 1.20	25.50 ± 1.20
	21	30.15 ± 1.50	29.20 ± 1.00	27.10 ± 1.30	25.20 ± 1.15
	28	30.15 ± 1.00	29.45 ± 0.50	26.50 ± 1.15	25.00 ± 0.50
MCH (pg)	7	35.48 ± 4.50	50.55 ± 4.21	65 ± 3.26	70.23 ± 3.88
	14	34.33 ± 4.25	50.45 ± 4.15	65.44 ± 3.50	72.46 ± 3.40
	21	34.30 ± 4.15	52.35 ± 4.30	67.23 ± 2.77	76.54 ± 2.67
	28	33.48 ± 4.68	54.33 ± 4.15	70.15 ± 1.20	77.55 ± 3.44
MCV	7	82.33 ± 4.46	103.96 ±0.97	183.64±2.34	205.65± 5.66
	14	82.14 ± 4.26	105.4 ± 0.92	187.56±2.66	202.33±5.78
	21	83.11 ± 4.33	105.86 ±0.96	190.33±2.87	210.56± 4.78
	28	85.23 ± 4.12	108.66 ±0.99	195.46±3.48	239.45±3.57

Comment [A6]: ANOVA not reflected on the values.

Table 1: Showing variations in haematological parameters of *Channapunctatus* exposed to CrO_3 for different durations.The data are represented in mean ± SEM.

Comment [A7]: Table label should be on top of each table.

Comment [A8]: Of how many determinations?

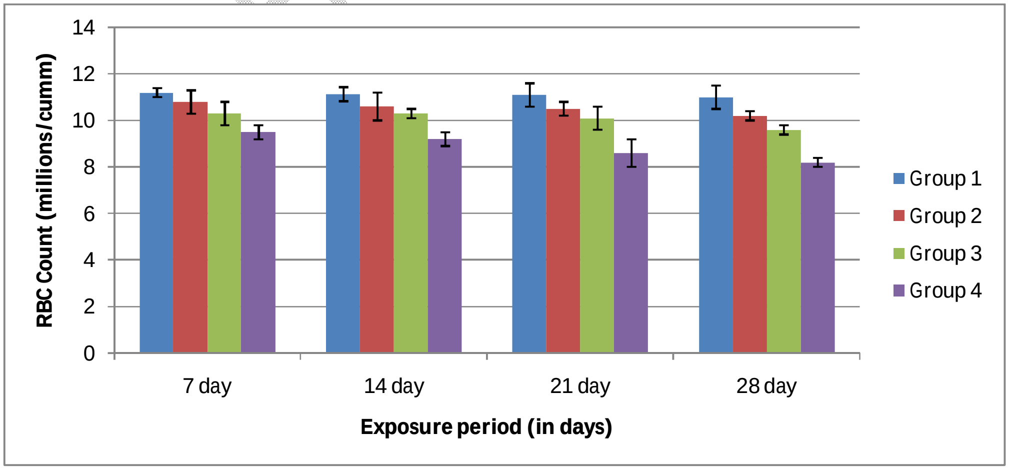


Fig. 1.~~The graph represents~~RBC count values~~in blood~~ of *C. punctatus*~~in all~~ exposed to varying amount of CrO$_3$~~groupsafter 28 days~~. The values are represented in mean ± SEM.

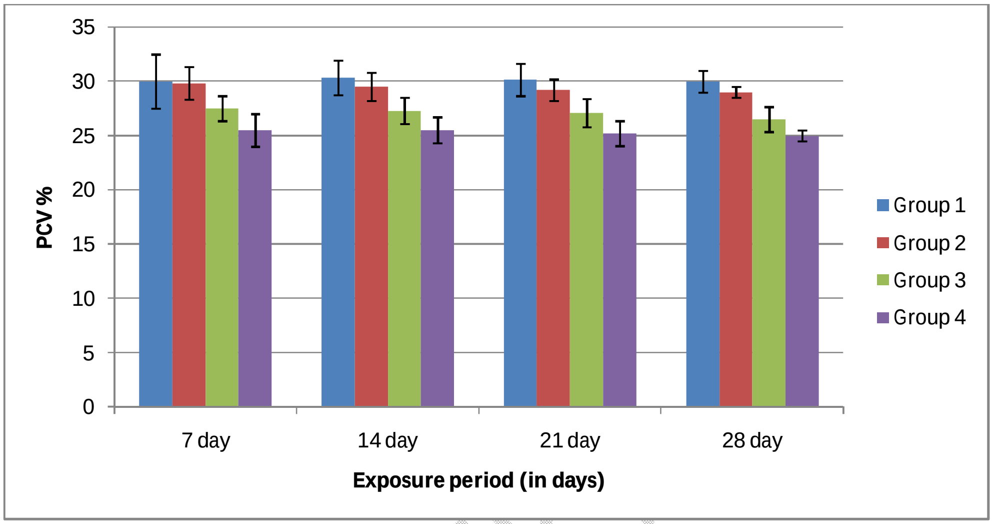
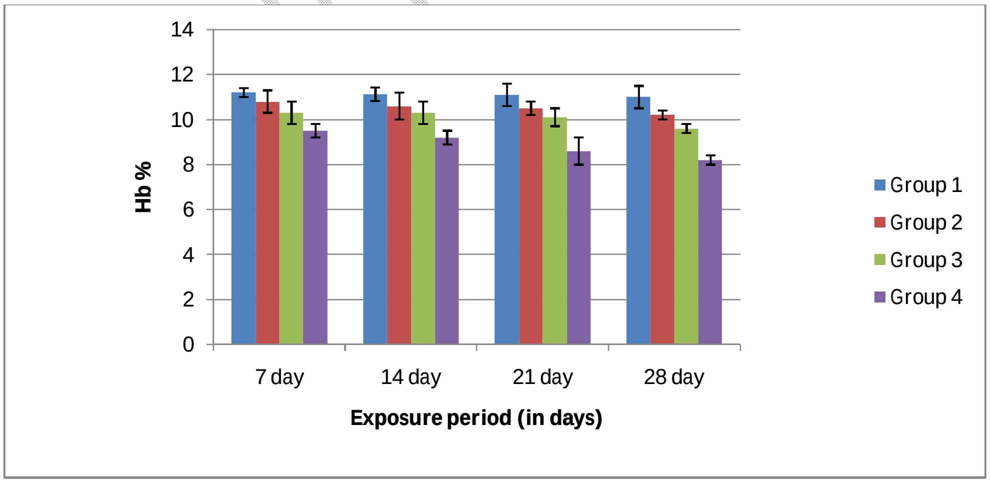


Fig. 2. The graph represents PCV value in blood of *C. punctatus* in all exposed groups after 28 days. The values are represented in mean ± SEM.



Comment [A9]: But there are results for after 7, 14 and 21 days. So use the above correction for all.

Fig. 3. The graph represents Hb% value in boold of *C. punctatus* in all exposed groups after 28 days. The values are represented in mean ± SEM.

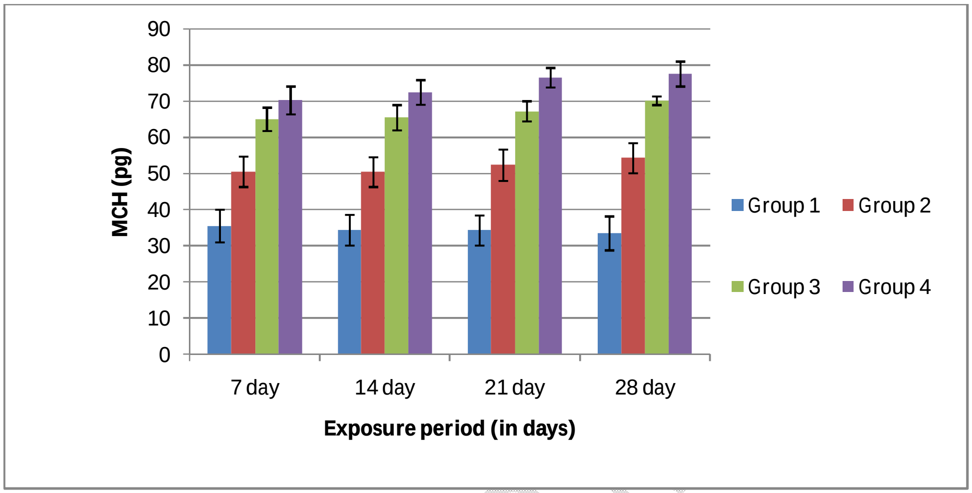


Fig. 4. The graph represents MCH value in boold of *C. punctatus* in all exposed groups after 28 days. The values are represented in mean ± SEM.

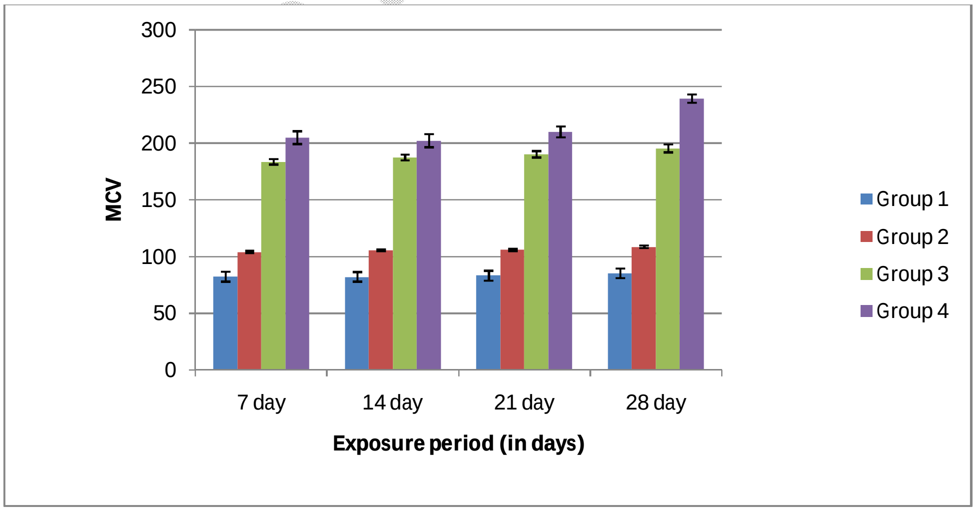


Fig. 5. The graph represents MCVvalue in boold of *C.punctatus*in all exposed groupsafter 28 days. The values are represented in mean ± SEM.

Haematology is used as an indicator of fish health conditions in a range of fish species in order to detect different stress situations such as illness, hypoxia, exposure to metals and pollutants, etc. (Blaxhall&Daisley, 1973). The results of this research show that fish erythrocytic parameters are affected by hexavalent chromium. According to Javed et al. (2016), the decrease in haematological values might potentially be attributed to heavy metals (pollutants) altering the structure of RBCs, which prevented more oxygen molecules from binding to Hb, decreased RBC oxygen carrying capacity, decreased Hb levels, and even caused anaemia. According to past studies (Karuppasamy, 2000; Zutshi et al., 2010), heavy metals may damage erythrocytes, which would explain the significant drop in haemoglobin content. Because of the low haemoglobin level, the fish's vital organs are not getting enough oxygen. Degradation of the erythrocytes and a persistent drop in haemoglobin concentration in fish exposed to heavy metals may be regarded as pathological conditions that limit oxygen delivery (Pamila et al., 1991). To further pinpoint the anaemic status of these experimental fish groups, hematocrit measurement was employed. Both haemoglobin levels and Hct% significantly decreased after exposure to the heavy metal chromium, which clearly suggests a hemodilution mechanism that may be brought on by gill injury or poor osmoregulation. In *Labeorohita* exposed to chromium, the mean MCV and MCH levels significantly altered as well (Venkatachalam& Natarajan, 2014). A similar discovery in *C. carpio* after exposure to cadmium was reported (Koyama & Ozaki, 1984). The fact that the values for Hb%, MCH, and hematocrit in *Channapunctatus* exposed to both copper and chromium declined drastically ~~in 1995~~ implies that metal causes acute anaemia under risky circumstances, according to Singh (1995).

Comment [A10]: What could be the mechanism behind increase in MCV and MCH recorde3d in this study?

Conclusion

Pollutants ~~will~~ pose a major danger to aquatic ecosystems. This research shows a clear correlation between Cr (VI) content and fish population survival. Freshwater fish species are more vulnerable to Cr (VI) in comparison. Haematological measures ~~should~~can be used to measure chromium since they may be helpful in the early diagnosis of fish health conditions before they worsen. It is hoped that, ~~T~~the research community will be benefit from the~~assisted in~~

Comment [A11]: Percentage mortality during this experiment, was not recorded.

Comment [A12]: ...to other aquatic organism???

~~gathering cutting-edge~~ information on the ecotoxicology and risk assessment of hazardous chromium by the scientific evidence offered in this study, which provides a basis for comprehending the possible influence of chromium on a fish species' haemoglobin. Furthermore, ~~I~~it has been shown that fish exhibit a broad spectrum of chromium-related impacts on their histology, physiology, biochemistry, enzymology, haematology, and genetic make-up. The same negative effects that chromium has on indicator organisms (fish) will ultimately reach humans when it enters the food chain via water and food. Thus, one of the main approaches to solving the issue is to prevent chromium poisoning of aquatic habitats from happening again.

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