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Toxicity of Pentachlorophenol Exposure on Male and Female *Heteropneustes fossilis* Investigated Using NMR-Based Metabolomics Approach

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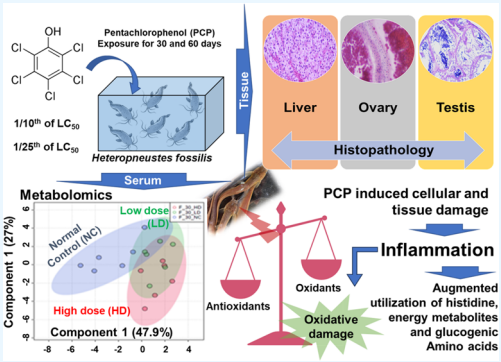
ABSTRACT: Pentachlorophenol (PCP) is one of the most common chlorophenols utilized in numerous industrial processes, including the production of dyes, pesticides, wood preservatives, disinfectants, antiseptics, and medicines because it has fungicidal and bactericidal characteristics. Previous studies on catfish (*Heteropneustes fossilis*) revealed that PCP acts as a potent endocrine disruptor and also causes behavioral changes in a concentration-dependent manner. However, the toxicological effects of PCP have not been compared between male and female catfish. The present study aims to investigate the toxic effects of PCP on catfish through histopathological changes, oxidative stress, and serum metabolomics after 60 days of exposure. Chronic exposure to sublethal concentrations of PCP resulted in significant histopathological alterations in the liver and gonad, including leukocyte infiltration, hepatocyte degeneration, follicular layer dissolution, and abnormal sperm distribution. Increased levels of lipid peroxidation and hydrogen peroxide, along with decreased antioxidant enzyme activity, were observed in PCP-exposed groups. A ^{1}H NMR-based metabolomics approach was employed to investigate the toxic effects of PCP on catfish serum, revealing alterations in various metabolites, including amino acids, organic acids, glucose, cholesterol, and neurotransmitters, in a dose-dependent manner. Multivariate partial least-squares discriminant analysis (PLS-DA) identified metabolic changes associated with oxidative stress, disruption in hormone synthesis and reproduction, disturbance in osmoregulation and membrane stabilization, energy metabolism disorder, amino acid metabolism disorder, and neurotransmitter imbalance in PCP-exposed catfish. This study demonstrates the efficacy of metabolomics in elucidating the toxicity and underlying mechanisms of wood preservatives like PCP, providing valuable insights for risk assessment in toxicology research. Overall, these findings contribute to our understanding of the toxicological effects of PCP exposure on aquatic organisms and highlight the potential of histology, oxidative stress, and metabolomics in assessing environmental contaminants' risks.



INTRODUCTION

Chlorophenols due to their adverse toxicity and carcinogenic potential are categorized as priority pollutants by regulatory agencies like EU's environmental legislation (Council Directive 2455/2001/ECC, 2001) and United States Environmental Protection Agency (U.S. EPA, 1999). Pentachlorophenol (PCP) is one of the most common chlorophenols utilized in numerous industrial processes, including the production of dyes, pesticides, wood preservatives, disinfectants, antiseptics, and medicines because it has fungicidal and bactericidal characteristics. PCP exposure can have harmful effects on human health including skin irritation, respiratory problems, and potential carcinogenicity. PCP is also listed as a priority pollutant and is a possible human carcinogen. PCP can also significantly negatively impact aquatic ecosystems by inhibiting photosynthesis and disrupting the food chain. Due to their sensitivity to water contamination, fish have been extensively examined in ecotoxicological studies as sentinels to determine the health state of the environment.[1−3] Fish can actually effectively absorb and store pollutants in their tissues as well as produce quantifiable reactions to toxic assaults. Even worse, those at the top of the food chain, such as humans, are particularly at risk from the consumption of contaminated aquatic species due to bioaccumulation. Therefore, measures such as monitoring and regulating the use and discharge of PCP are necessary to protect human health and the environment. Water treatment processes can also be implemented to remove PCP from contaminated water sources.

Heteropneustes fossilis (commonly known as catfish), serves as a valuable model organism for investigating the harmful

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effects of environmental pollutants/contaminants on aquatic ecosystems. Male and female organisms may respond differently to toxic exposures due to variations in physiology, metabolism, and hormonal regulation. Therefore, understanding how PCP affects the male and female population of *H. fossilis* species will not only improve our understanding of how PCP affects different biological systems of this species but also help inform conservation efforts and regulatory measures aimed at reducing environmental contamination.

The metabolism of PCP involves the enzyme nitroreductase, which converts PCP into xenobiotic metabolites, promoting the production of DNA adducts. PCP inhibits nitroreductase and increases β-glucuronidase activity.[4,5] Oxidative dichlorination of PCP in the liver results in the production of tetrachlorohydroquinone (TCHQ) and semiquinone,[6] along with hydrogen peroxide (H_2O_2). This metabolic process can lead to the generation of superoxide radicals under specific physiological conditions. The dissociation of PCP during mitochondrial oxidative phosphorylation[7] induces the production of reactive oxygen species (ROS), contributing to DNA and cell component damage.[8] Therefore, a current strategy is to use "-omics" techniques, which serve as useful tools for elucidating the intricate impact of chemical pollutants/contaminants on the health of different organisms. These techniques enable the simultaneous evaluation of a wide range of biomolecules and help elucidate the molecular events or metabolic pathways affected in response to environmental perturbations, disease, and/or genetic/epigenetic changes.[9,10]

The purpose of the present study is to investigate the histopathological aberrations, oxidative stress, and serum metabolomics alterations induced by chronic PCP exposure in male and female catfish *H. fossilis*. Metabolomics involves quantitative and comparative analysis of small endogenous small metabolites (with molecular weight less than 1 kDa) present in biological systems (such as cells, tissues, biofluids, or entire organisms) and provides insights into the intricate toxicological pathways directly or indirectly affected by environmental pollutants/contaminants.[11,12] Proton nuclear magnetic resonance (^{1}H NMR) spectroscopy stands as the leading analytical technique for high-throughput metabolic profiling owing to its numerous advantages including non-destructiveness (allowing samples to remain intact for further analysis), requires little to no separation, and provides quantitative information for multiple circulatory metabolites containing protons such as amino acids (AAs), organic acids, fatty acids, polyamines, and sugars/carbohydrate molecules.[13−16] Routinely, ^{1}H NMR spectroscopy is used in combination with chemometric pattern recognition approaches for metabolomics data analysis.[17]

RESULTS

PCP is commonly used as a pesticide and can bioaccumulate in fish, posing a risk to humans who consume contaminated fish. The present study aims to explore the biometric indicators, histopathological changes, oxidative stress levels, and serum metabolomics alterations induced by chronic PCP exposure in catfish. By investigating these parameters on this model organism, we seek to understand the toxicological mechanism of prolonged PCP exposure. The various results are summarized below.

Biometric Indicators: Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI). Gonadosomatic index (GSI), which reflects the ratio of ovary weight to body weight, serves as a reliable indicator of ovarian recrudescence, showing significant seasonal variations. It is low during the gonad-inactive phase and increases during the active phase, peaking before spawning, as evidenced by increase in the control groups. Lowest GSI mean values were observed in low-dose PCP-exposed catfish. For both the gonads (ovary and testis) of catfish *H. fossilis* exposed to PCP for 60 days, there is a decrease in the gonadosomatic index with respect to control (Figure 1).

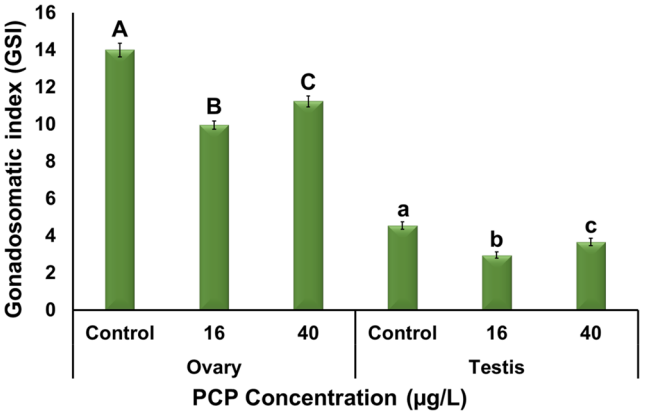

Figure 1. Gonadosomatic index (GSI) of female and male *H. fossilis* exposed to PCP for 60 days. Data were expressed as mean ± standard error in mean (SEM) of eight different preparations and compared using one-way analysis of variance (ANOVA) statistics ($p < 0.001$) followed by Tukey's test ($p < 0.05$). The groups with different uppercase and lowercase letters represent the comparison among the different concentrations within ovary and testis.

Hepatosomatic index (HSI) reflects the ratio of liver weight to body weight and serves as an indicator of the liver function in vitellogenesis. During gonadal maturation, hepatocytes play a vital role in producing vitellogenin (Vtg) and zona radiata proteins (Zrp). These proteins are then transported to the ovaries via receptor-mediated pinocytosis through the bloodstream.[22] The HSI thus provides a measure of liver involvement in supporting reproductive processes. HSI mean values of high-dose PCP-exposed catfish were the lowest. For the liver of both female and male catfish *H. fossilis* exposed to PCP for 60 days, there is a dose-dependent decrease in the hepatosomatic index with respect to control (Figure 2).

Histopathology. *Gonad.* Gonado-histopathology changes serve as valuable biomarkers for assessing contaminant-induced reproductive consequences in fish, allowing for the examination of cellular structural changes before clinical manifestations occur. This method has been an effective means of evaluating the impacts of contaminants on reproductive health. Histologically, catfish ovaries appear to be dominated by vitellogenic oocytes which indicated that the gonad has been at prespawning stage of the reproductive cycle, containing yolk granules that frequently fill the entire center of oocyte, coated by demarcated zona radiata, granulosa, and surface epithelium (Figure 3A). The exposure to PCP led to significant changes at the follicular epithelium level in the ovary, with the extent of these changes varying based on the dose and duration of exposure. In low-dose PCP-exposed ovary (16 μg/L), we have observed dissolution of follicular layer, disintegration of the granulosa cell structure, increase in the interfollicular space (Ifs), and oocyte wall was either vacuolated or detached from

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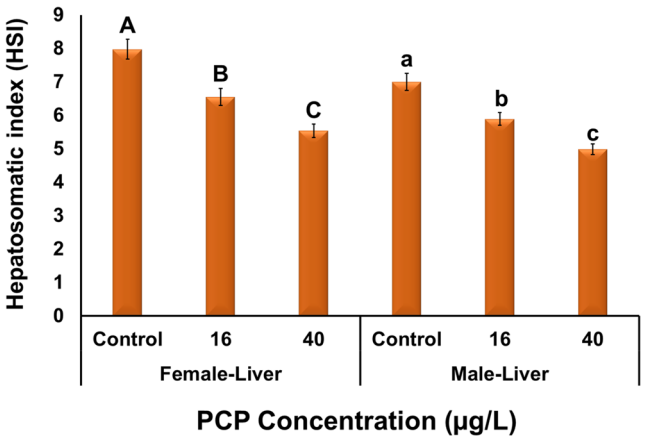

Figure 2. Hepatosomatic index (HSI) of female and male *H. fossilis* exposed to PCP for 60 days. Data were expressed as mean ± SEM of eight different preparations and compared using one-way ANOVA statistics (p-value <0.001) followed by Tukey's test (p-value <0.05). The groups with different uppercase and lowercase letters represent the comparison among the different concentrations within the liver of female and male catfish.

the oocytes (Figure 3B). In high-dose-exposed ovary (40 µg/L), we have observed dissolution of the follicular layer, and the oocyte wall was either vacuolated or detached from the oocytes extensively (Figure 3C). Histologically, the control testis of *H. fossilis* shows a well-defined arrangement of its cells. Interlobular connective tissue (ICT) containing Leydig cells envelops the seminiferous tubules. Within each compartment, the primary spermatogonia, secondary spermatogonia, Sertoli cells, lobule walls, and a central area called the lobular lumen—which is primarily occupied by spermatozoa, are all well organized (Figure 3D). In the testis derived from catfish exposed to a low-dose PCP (16 µg/L), we observed spermatozoa clumping, aberrant spermatozoa distribution, interstitial tissue fibrosis, necrotic spermatogonia, and intertubular vacuolation (Figure 3E). The testis derived from catfish exposed to a high-dose PCP (40 µg/L) showed significant spermatozoa clumping, aberrant spermatozoa distribution, and interstitial tissue fibrosis (Figure 3F).

Liver. The liver, serving as the primary organ for a multitude of crucial metabolic processes, plays a central role in maintaining physiological homeostasis in organisms. In teleosts, a class of bony fish that includes many familiar species, the liver is particularly susceptible to the impact of environmental contaminants. Exposure to PCP can lead to noticeable alterations in the histoarchitecture, biochemistry, and overall physiology of the liver. During the prespawning phase, the liver of catfish typically displays a large spherical nucleolus and varying amounts of dispersed and peripheral heterochromatin. Hepatocytes, which are situated among blood capillaries called sinusoids, form cord-like structures known as hepatic cell cords. The lumen of sinusoids primarily contains erythrocytes, and Kupffer cells are observed resting on the luminal surface of the sinusoidal endothelium (Figure 4A). Catfish exposed to a low concentration of PCP generally showed lateral shifting of the nuclei, presence of pycnotic cell and kupffer cell, karyolysis, and dilation in sinusoids (Figure 4B). In catfish exposed to high concentration of PCP, we observed all of the above abnormalities but at a severe rate, which were reported in low dose with extensive fibrosis in blood vessels (Figure 4C).

Nonenzymatic Oxidative Stress Markers. In both humans and animals, the journey of PCP undergoes a transformative path postabsorption, involving crucial processes such as conjugation, hydrolysis, and reductive dichlorination.[37,38] PCP engages in complex redox cycling mechanisms, ultimately transforming into highly lethal entities tetrachloro-*p*-benzoquinone (TCBQ) and tetrachlorohydroquinone (TCHQ). The conversion initiates a cascade of events involving the generation of semiquinone radicals, catalyzed by oxidation of TCHQ and/or reduction of TCBQ facilitated by nicotinamide adenine dinucleotide + H (NADH). These radicals react with molecular oxygen, producing hydrogen peroxide (H_2O_2) and superoxide, further potentiated by

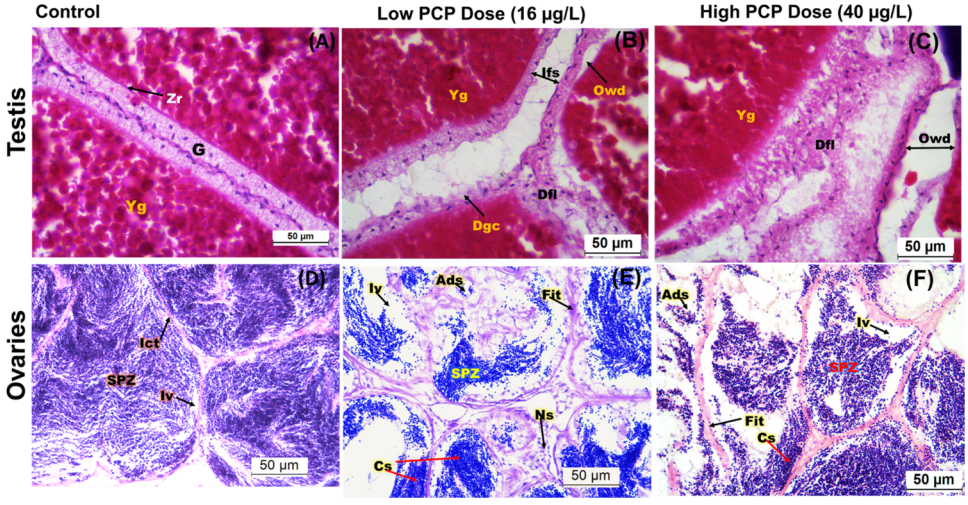

Figure 3. Histopathological alterations in the gonad of *H. fossilis* exposed to PCP for 60 days: (A) control ovary; (B) low-dose ovary (16 µg/L); (C) high-dose ovary (40 µg/L); (D) control testis; (E) low-dose testis (16 µg/L); and (F) high-dose testis (40 µg/L). Yolk granules, Yg; granulosa, G; zona radiata, Zr; dissolution of follicular layer, Dfl; disintegration of the granulosa cell structure, Dgc; increase in the interfollicular space, Ifs; oocyte wall was either vacuolated or detached from the oocytes, Owd; spermatozoa, SPZ; interlobular connective tissue, Ict; intertubular vacuolation, Iv; clumping of spermatozoa, Cs; abnormal distribution of spermatozoa, Ads; fibrosis of interstitial tissue, Fit; necrotic spermatogonia, Ns. Scale bar = 50 µm, magnification = 40×.

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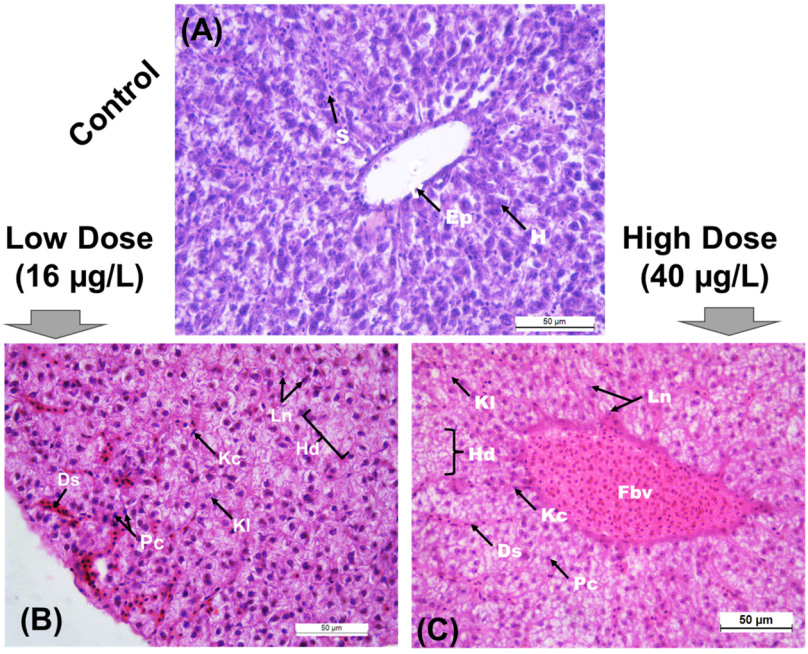

Figure 4. Histopathological alterations in the liver of *H. fossilis* exposed to PCP for 60 days: (A) control; (B) low dose (16 μg/L); (C) high dose (40 μg/L). Hepatocytes, H; kupffer cell, Kc; lateral nuclei, Ln; pycnotic cell, Pc; karyolysis, Kl; hepatocyte degeneration, Hd; dilation in sinusoids, Ds; fibrosis in blood vessel, Fbv. Scale bar = 50 μm, magnification = 40×.

activation of transition metals, collectively contributing to cellular damage.[37,39] In tissue homogenates from control and PCP-treated catfish, nonenzymatic indicators of cellular oxidative stress were evaluated. The results demonstrated that PCP altered nonenzymatic characteristics in catfish tissues in a dose-dependent manner. H_2O_2 and lipid peroxidation (LPO) levels were higher than in the control group. The reduced glutathione (GSH) level was substantially lower than that of the control (Figure 5).

Antioxidant Status. The toxic effect of PCP on the antioxidant potential of liver and gonad cells was examined to investigate the involvement of the antioxidant system in PCP-induced toxicity. ROS produced by xenobiotics are significant in cell damage in a variety of tissues. Antioxidant enzymes play an important role in cellular defense against ROS. The activities of various antioxidant enzymes were found to be significantly reduced in PCP-exposed catfish in a dose-dependent manner (Figure 5, uppercase letters denote a significant difference in liver tissue, whereas numerals and lowercase letters denote significant difference in ovary and testis of catfish *H. fossilis*).

Serum NMR Spectral Assignment. NMR-based serum metabolomics was employed to compare the quantitative levels of serum metabolites and understand the metabolic pathways underlying the affected in PCP-exposed catfish. Figure 6 shows the stack plot representation of one-dimensional (1D) ^{1}H Carr−Purcell−Meiboom−Gill (CPMG) NMR spectra of serum samples obtained from the control and PCP-exposed female (Figure 6A) and male (Figure 6B) catfish. The spectral peaks corresponding to ^{1}H NMR signals of various circulatory metabolites are labeled in the figure. The serum spectra revealed signals from (a) amino acids such as alanine, glycine (Gly), lysine, valine, proline isoleucine, leucine, glutamate (Glu), glutamine, methionine, phenylalanine (Phe), histidine, and tyrosine, (b) energy metabolites such as glucose (Gls), lactate, and creatine, (c) lipid and membrane metabolites such as choline, glycerol-phosphocholine (GPC), (d) lipoproteins including very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL), and (e) some other metabolites (like formate, acetate, 3-hydroxybutyrate (3HB), citrate, succinate, acetone, O-acetyl carnitine (OAC), and *N*-acetylglycoproteins (NAG)).

Multivariate Discriminatory Analysis and Metabolic Alterations in PCP-Exposed Catfish. Multivariate discriminatory analysis was carried out to compare the serum metabolic profiles of PCP exposed and the control study groups of catfish. To overview the quality of the NMR-based metabolomics data matrix and identify potential outliers, principal component analysis (PCA) was employed. The PCA score plots (not included in this paper) showed only slight discrimination between PCP-exposed and control study groups. Therefore, partial least-squares discriminant analysis (PLS-DA) was considered primarily to evaluate the metabolic disparity study groups. As shown in Figure 7A,C, the PCP-exposed and control catfish serum samples formed distinct clusters and these clusters were also exquisitely separated and orientated differently in PLS-DA score plots, indicating significant differences in metabolic profiles between the study groups compared. The PLS-DA model further helped to index the discriminatory metabolites based on the VIP statistics criterion, considering metabolites with VIP scores greater than 1.0 as significant discriminators (Figure 7B,D). These discriminatory metabolites were then evaluated for statistical significance using the Mann−Whitney univariate test, with a p-value of <0.05 as cutoff threshold for statistical significance. Careful evaluation led to the identification of 10 discriminatory metabolites showing statistically significant differences between the study groups.

For PCP-exposed female catfish, compared with controls, the sera were characterized by (a) increased levels of amino acids, such as alanine, at low doses; and (b) decreased levels of histidine, valine, glutamate, leucine, pyruvate, 3-HB, citrate,

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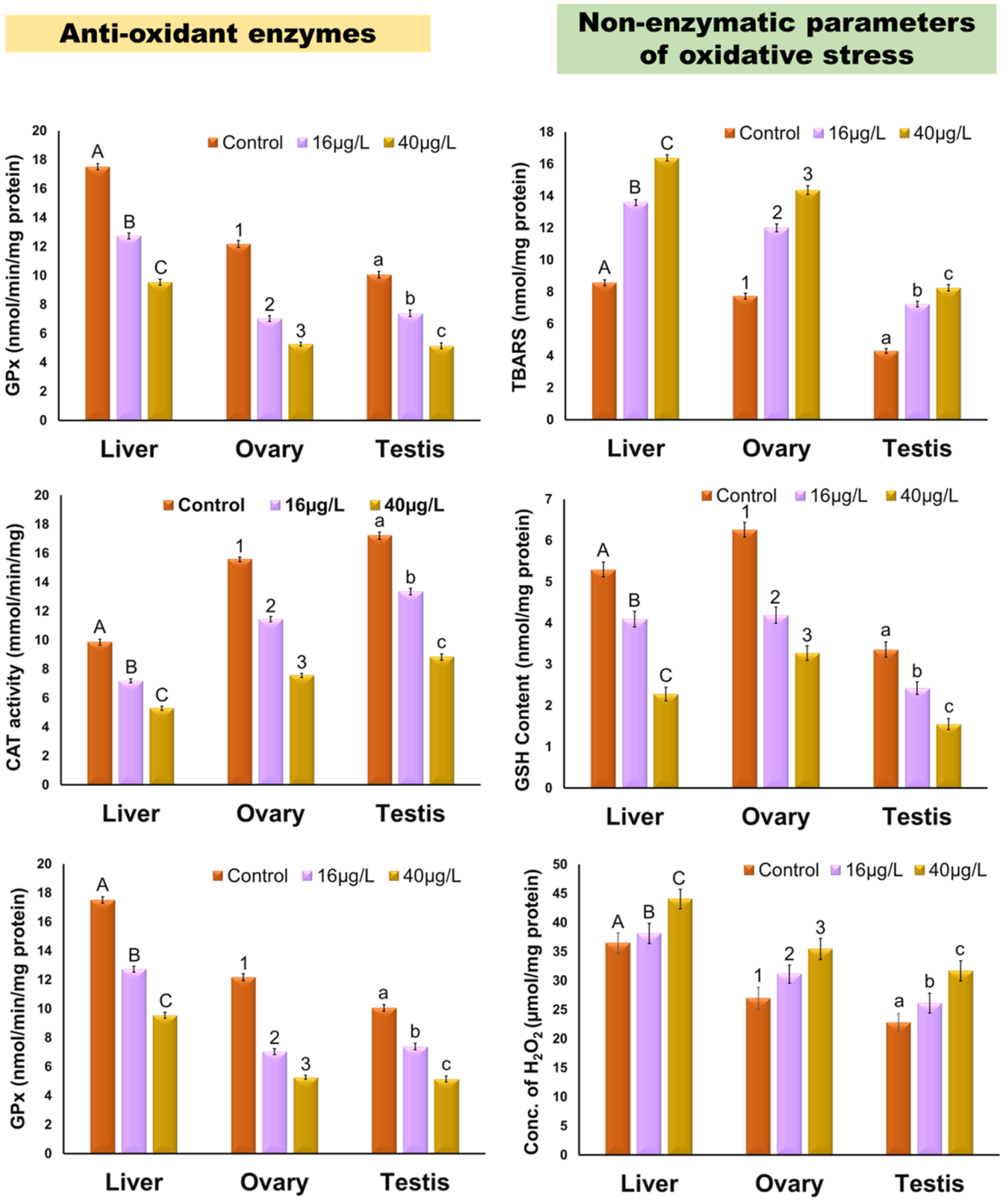

Figure 5. PCP-induced changes in the activities of key antioxidant (AO) enzymes and oxidative stress markers upon 60 days of exposure in the catfish *H. fossilis*. Left and right panels, respectively, show the changes in the activities of key antioxidant enzymes (CAT, SOD, GPx) and nonenzymatic parameters of oxidative stress (GSH, LPO, H_2O_2) in catfish liver and gonad tissue samples. Data were expressed as mean ± SEM of eight different preparations and compared using one-way ANOVA statistics (p-value <0.001) followed by Tukey's test (p-value <0.05). The uppercase and lowercase letters/numerals represent the comparison among the different concentrations within liver, ovary, and testis. Abbreviations: CAT, catalase; H_2O_2, hydrogen peroxide; GSH, reduced glutathione; LPO, lipid peroxidation; SOD, superoxide dismutase; and GPx, glutathione peroxidase.

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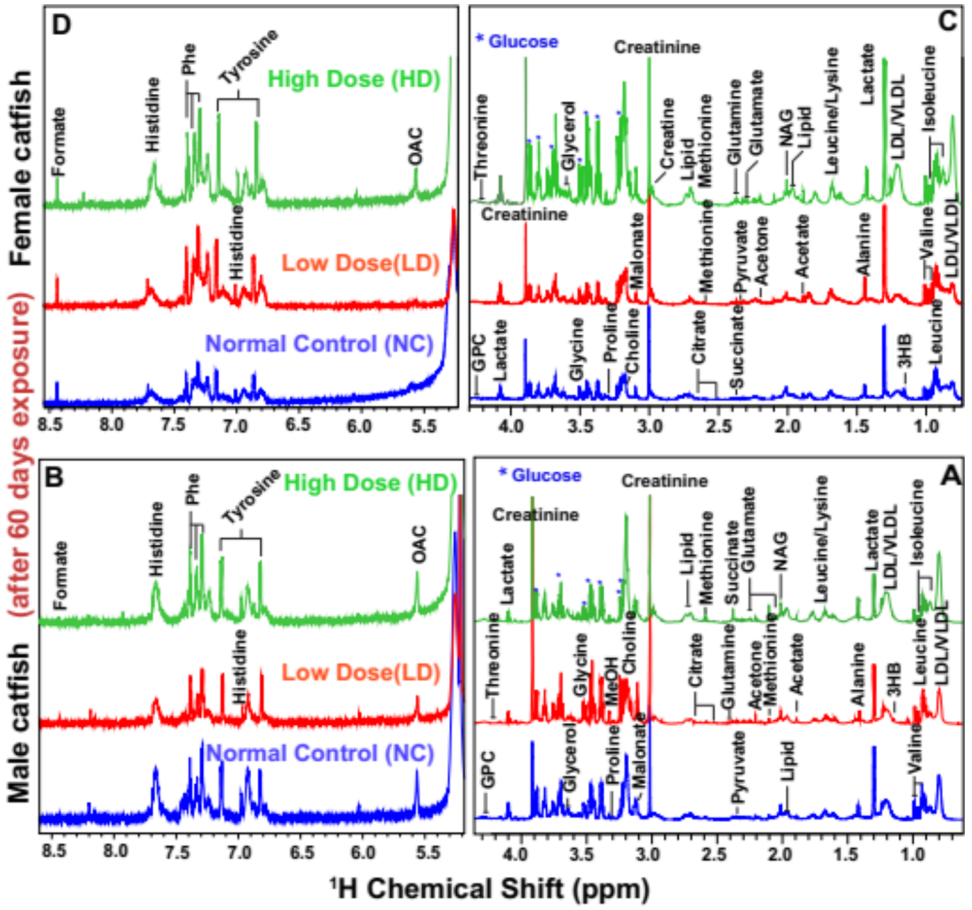

Figure 6. Stack plot representation of ^{1}H 1D CPMG NMR spectra of serum samples obtained from male (A, B) and female (C, D) *H. fossilis* catfish after 60 days of PCP exposure, compared with a normal control (NC). The assignment of NMR peaks corresponding to specific serum metabolites is annotated. The abbreviations used are 3-HB: 3-hydroxybutyrate; GPC: glycerophosphocholine; VLDL/LDL: very low/low-density lipoproteins; NAG: *N*-acetylglycoproteins; OAC: O-acetyl carnitine; Phe: phenylalanine.

and lactate. For PCP-exposed male catfish compared with controls, the sera showed decreased levels of valine, leucine, glucose, and glycine. Representative box-and-whisker plots illustrating the relative signal intensity for these potential metabolic markers are shown in Figure 8A,C. Additionally, multivariate analysis based on the PLS-DA model, showing the intervention effect of PCP treatment (for 30 days) on both female and male *H. fossilis* in comparison to control *H. fossilis*, is included in the electronic Supporting Information (ESM, see Figure S1). This analysis also revealed serum metabolic changes in male and female catfish exposed to PCP for 30 days.

Metabolic Response to PCP Exposure. 2D score plots clearly revealed that the metabolic perturbations in both male and female catfish are more aberrant in high-dose (HD) catfish compared to low-dose (LD) catfish with respect to the normal control (NC) group. However, the degree of alterations in serum metabolic profiles was different in both groups.

Pathway Analysis Using MetaboAnalyst. The serum metabolic features significantly altered in male and female catfish exposed to PCP (as per Figure 8) were further used to explore the associated metabolic pathways, and the results are summarized in Figure 9. Figure 9A shows the metabolic pathways affected in PCP-exposed male catfish, and this has been generated using the list of key metabolites: histidine, lactate, valine, leucine, acetate, citrate, acetone, and O-acetyl carnitine. As evident, the male catfish exposed to PCP for 60 days causes disturbances in citrate cycle (tricarboxylic acid (TCA) cycle), glyoxylate and dicarboxylate metabolism, histidine metabolism, pyruvate metabolism, glycolysis/gluconeogenesis, and branched-chain amino acids (BCAAs) (isoleucine, leucine, and valine) biosynthesis.

Figure 9B shows the metabolic pathways affected in PCP-exposed female catfish, and this has been generated using the list of key metabolites: valine, 3-hydroxybutyrate (3HB), lactate, glycerophosphocholine (GPC), citrate, acetone, threonine, cholesterol, and isoleucine. As evident, the female catfish exposed to PCP for 60 days causes disturbances in citrate cycle (TCA cycle), primary bile acid metabolism, glycerophospholipid metabolism, glyoxylate and dicarboxylate metabolism, steroid biosynthesis, and branched-chain (valine, leucine, and isoleucine) biosynthesis. The key metabolic pathway disturbances revealed by serum-based metabolomics analysis are also summarized in Figure 9C and are explicitly discussed in the following section.

DISCUSSION

PCP stands out as an environmentally persistent and bioaccumulative compound, contributing to fatal toxicity in aquatic species. Its deleterious effects extend across various physiological and reproductive aspects, making it a concerning pollutant for aquatic ecosystems. One of the primary targets of PCP toxicity is the liver and gonad, where it induces damage,

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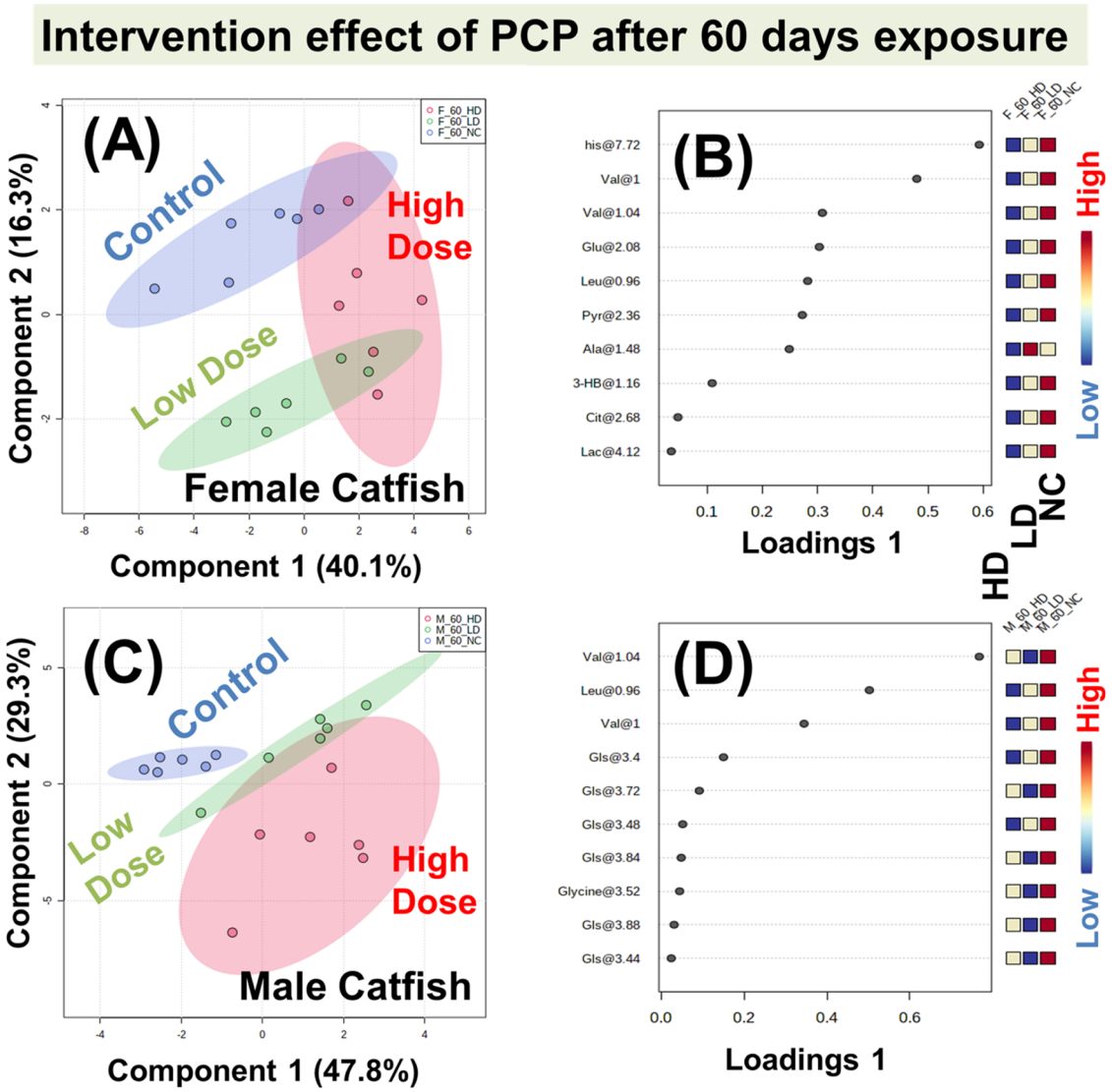

Figure 7. (A, C) Two-dimensional (2D) score plots were derived from partial least square-discriminatory analysis (PLS-DA) model analysis involving normalized spectral features of PCP-treated HD, LD, and NC; female and male catfish, *H. fossilis* for 60 days of PCP exposure. (B, D) VIP plot displays top 10 metabolite variation (higher or low) in serum of PCP-treated female and male *H. fossilis* in comparison to the NC *H. fossilis* for 60 days of PCP exposure.

leading to disruptions in normal physiological functions.[18,19] Additionally, PCP exposure elevates respiratory stress and mixed-function oxygenase activity, indicating interference with respiratory processes and detoxification mechanisms in aquatic organisms.[20] Furthermore, PCP has been documented to interfere with sexual maturation and reproduction in aquatic species. This disruption often manifests as adverse effects on gonadal development, fertility, and overall reproductive success.[19,21,22] PCP impact on reproductive health has been observed not only in vertebrates but also in certain invertebrates, affecting their energy metabolism by impeding the synthesis of adenosine triphosphate (ATP), a crucial energy carrier in cells.[23−25] In addition to its direct ecological impact, the International Agency for Research on Cancer (IARC) has classified PCP as a probable Group 2B carcinogen in 2004.[18]

Considering the potential adverse effects of PCP exposure on both aquatic species and human health, it is important to understand the affected physiological, reproductive, and other metabolic processes due to chronic PCP exposure so that regulatory measures can be taken to protect the integrity of aquatic ecosystems. The present study is an effort in this direction to investigate the histopathological aberrations, oxidative stress, and serum metabolic alterations induced by chronic PCP exposure on male and female catfish.

PCP Induced Biometric and Histological Changes. Exposure to PCP resulted in significant histoarchitectural changes in the testes of catfish *H. fossilis*. These changes included spermatozoa clumping, deformed interlobular connective tissue, aberrant spermatozoa distribution, interstitial tissue fibrosis, necrotic spermatogonia, and intertubular vacuolation. These findings are consistent with previous research on male *Clarias gariepinus* exposed to 4-nonylphenol,[26] which reported similar effects, such as disrupted interlobular connective tissue, degeneration of spermatogonia cells, abnormalities in spermatozoa and increased interstitial fibrosis.

Additionally, exposure to di(2-ethylhexyl)-phthalate in male *C. gariepinus* caused intertubular vacuolation, inflammation, deformation, and degeneration of tubular epithelium and

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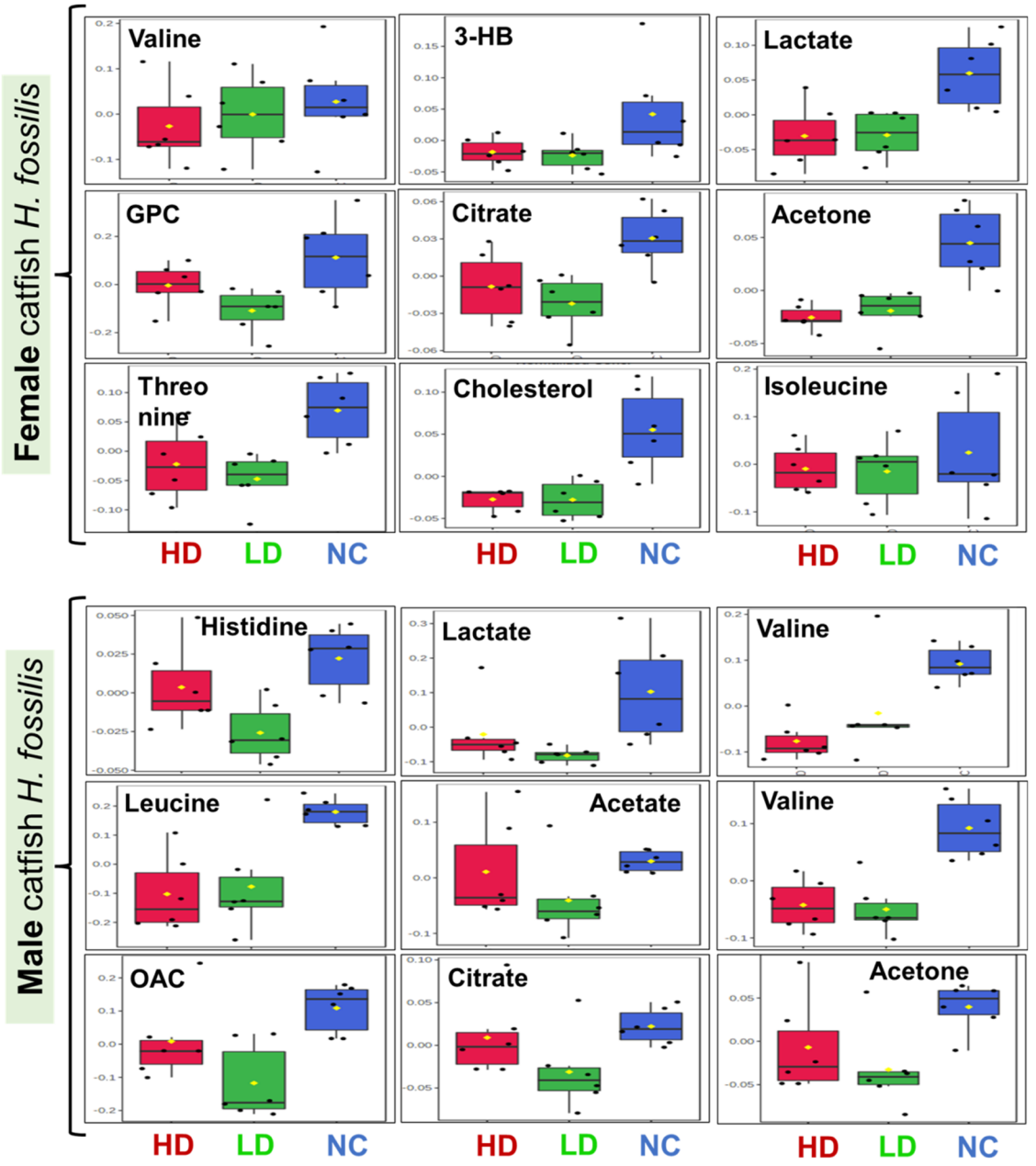

Figure 8. Metabolic effects of pentachlorophenol (PCP) treatment on female (upper panel) and male (lower panel) *H. fossilis* for 60 days. Representative box-cum-whisker plot showing relative variations in quantitative profiles of serum metabolites relevant in the context of the toxicology. In the box plots, the boxes denote interquartile ranges, the horizontal line inside the box denotes the median, and the bottom and top boundaries of boxes are 25th and 75th percentiles, respectively. Lower and upper whiskers are 5th and 95th percentiles, respectively, where normal control (NC), low dose (LD), and high dose (HD) groups.

seminiferous tubules, as well as tubular cell condensation.[27] These studies collectively demonstrate that pollutants can induce structural alterations in fish testis, affecting the size of seminiferous tubules and the overall tissue histology. Such impacts extend beyond reproductive organs, highlighting the broader effects of pollutants on tissue structure and function in aquatic organisms.

Compared to control groups, the PCP exposure for 60 days resulted in significantly decreased GSI parameters in both male and female catfish, albeit in a duration-dependent manner. Exposure to chlorophenols (CPs) over an extended period often results in a lower GSI in fish. For example, administration of 2,4-dichlorophenol (2,4-DCP) at concentrations exceeding 100 μg/L reduces GSI in male and female Chinese rare minnows (*Gobiocypris rarus*) over a 21-day period.[28,29] Similarly, at least three fish species were identified, including *Oreochromis niloticus*, *Chrysicthys nigrodigitatus*, and *Clarias gariepinus*, exhibit lower GSI after 60 days of PCP exposure.[28−30] Another study on sexually matured rare minnow (*G. rarus*) exposure to PCP over 28 days showed a significant decrease in GSI.[21] Histological analysis of catfish ovaries revealed the disintegration of oocytes and significant oocyte loss as the causes of GSI decline. Thus, GSI can serve as an end point for assessing endocrine disturbance, as supported by our current results and previous research.

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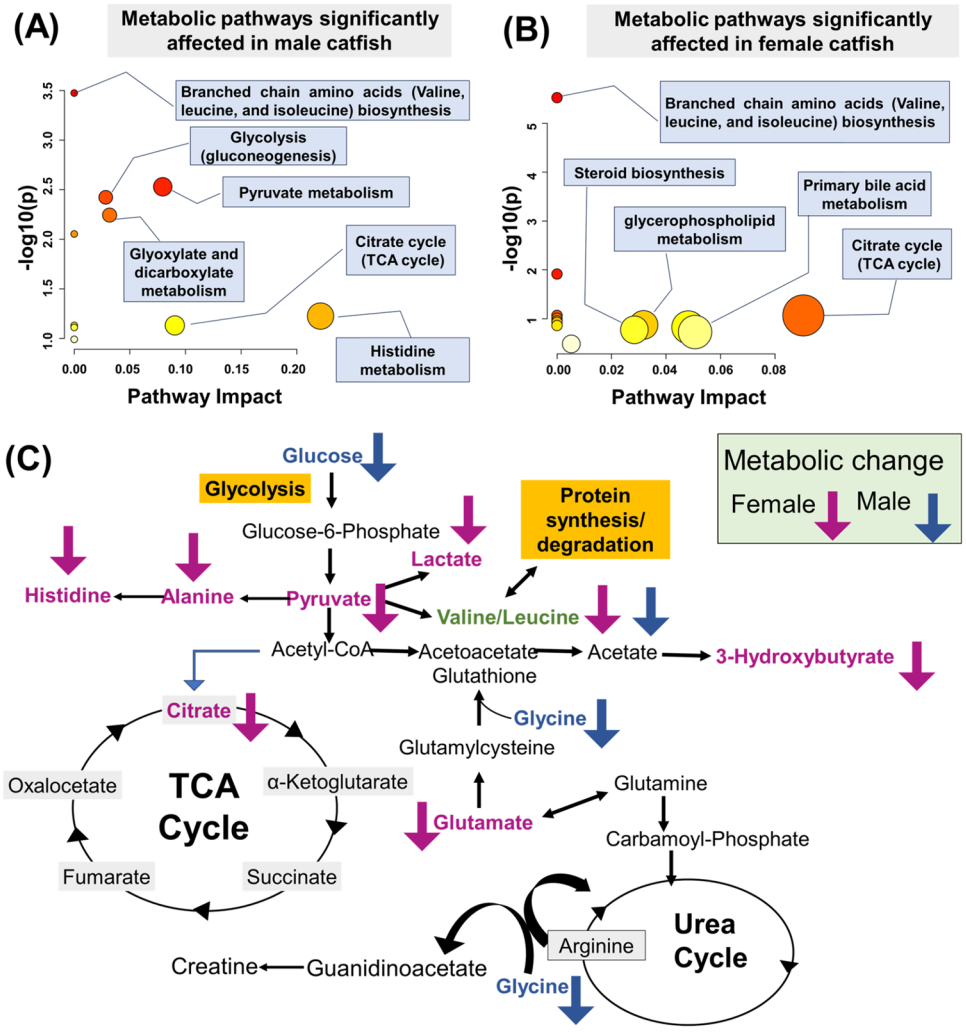

Figure 9. Metabolic pathway analysis performed using key metabolic changes observed for male (A) and female (B) catfish after 60 days of PCP exposure. (C) Scheme illustrating the major perturbed metabolic pathways in PCP-exposed catfish *H. fossilis* revealed by serum-based metabolomics analysis. TCA cycle represents the junction pathway of energy metabolism and amino acid metabolism. Arrows (↓↑) in different colors were used to represent the notable decrease/increase of metabolites in female (pink) and male (blue) of PCP-intoxicated catfish relative to healthy normal control catfish.

Compared to control groups, the PCP exposure for 60 days resulted in significantly decreased GSI parameters in both male and female catfish, albeit in a dose-dependent manner. Further, histological analysis of liver tissues from *H. fossilis* exposed to sublethal concentrations of PCP for 30 and 60 days during the study showed severe and varying effects, such as hepatocyte degeneration, hepatic cord disorganization, and cells with eccentric nuclei or lateral nucleus shifting. Similar findings were described by Selvanathan et al.,[31] who observed that hepatocytes ruptured with eccentric nuclei and the hepatic cords were severely disorganized. Hepatocyte separation and hepatic cord disarray are most likely caused by cell necrosis and structural protein degradation in the hepatocyte membrane.[32,33] The study also showed that exposure to PCP increased the number of Kupffer cells, which in turn might be triggered by the phagocytic activity of the sinusoidal cells. Similar results in fish exposed to several environmental toxicants.[34] With lysosomes involved in intracellular breakdown into smaller metabolic products, this increase in Kupffer cells may represent improved autophagy inside hepatic tissue, aiding in the clearance of stored PCP and its metabolites. Kupffer cell hyperplasia, which is a detoxifying defense mechanism and contributes to hepatic stress, may be correlated with the extent of hepatic tissue harm caused by 4-NP intoxication.[35] In addition, the liver showed the presence of pycnotic cells, karyolysis, and a high degree of leukocyte infiltration, sinusoidal dilatation, and fibrosis within hepatocyte blood vessels. Numerous researchers have observed similar results in fish exposed to different xenobiotics.[36,37] Blood vessel fibrosis is linked to a number of fish disorders,[38] and in the present study widespread incidence of fibrosis may be the consequence of energy loss during the detoxification process, which raises metabolic byproduct levels and causes blood vessels to aggregate.[39]

Our findings suggest that the initial estrogenic effects of PCP were overshadowed by its toxic actions, which manifested in a dose- and exposure-dependent manner. Atretic changes were observed at the cytoplasmic and follicular epithelium levels, including extensive loss of oocytes, leading to the formation of empty spaces, vacuolation, folding, and dissolution of the follicular layer. Additionally, there was disintegration of the granulosa cell structure, an increase in interfollicular space, and

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vacuolation or detachment of the oocyte wall. These alterations likely disrupted the endocrine functions of the follicular epithelium, resulting in inhibited or disturbed steroid production. Similar histopathological changes have been reported in other species exposed to PCP.[40,22] Previous studies have also noted PCP-induced apoptotic activity in both the testes and ovary of *H. fossilis*,[41] suggesting that the atretic changes observed may be triggered by cellular apoptotic mechanisms.

PCP Induced Oxidative Stress and Reduced Antioxidative Activity. The antioxidant glutathione (GSH) is essential for cells to defend against reactive oxygen species (ROS) and other oxidants, including lipid peroxides.[42] Cysteine (Cys), glycine (Gly), and glutamate (Glu) synthesize GSH by the enzymes glutamylcysteine synthetase and GSH synthase.[43] Glu regulates the synthesis of GSH by (1) preventing the inhibitory impact of GSH on γ-glutamylcysteine synthase and (2) facilitating the absorption of cystine into cells.[43] Recent findings have demonstrated that raising Glu levels increases GSH concentration and antioxidant ability in enterocytes.[44] Glu enhances the antioxidant potential and regulates the synthesis of antioxidant enzymes in carp enterocytes, including glutathione reductase (GR), glutathione peroxidase (GPx), and catalase (CAT).[44] According to the NMR-based metabolomics technique, the key precursor amino acids of reduced glutathione (GSH), i.e., Gly and glutamate (Glu), were significantly lower in the serum of *H. fossilis* catfish exposed toPCP-contaminated water. This suggests a change in the activity of oxidative stress enzymes, leading to oxidative damage in the catfish. This implies a potential dose-dependent increase in H_2O_2 levels in catfish *H. fossilis* exposed to PCP for 60 days during the prespawning phase of the reproductive cycle, highlighting the dynamic nature of PCP-induced oxidative stress.

Increased H_2O_2 levels and the heightened production of free radicals lead to a decrease in the antioxidant (AO) strength, which triggers oxidative damage to cellular constituents. This loss is attributed to the reduced activity of AO enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD), as well as diminished amounts of glutathione (GSH).[18] In our result, we observed a significant decrease in the gonad and liver of catfish exposed to PCP for 60 days with respect to AO enzymes (SOD, CAT, GSH). The compromised antioxidant defense systems create vulnerability, exposing tissues to oxidative damage, which is a crucial aspect of PCP toxicity. As a consequence of 60 days of PCP exposure, the LPO levels in the catfish significantly increased. Additionally, malondialdehyde (MDA), a byproduct of lipid peroxidation (LPO), emerges as a significant player in PCP-induced toxicity. Free radicals attack unsaturated fatty acids, initiating a chain reaction that leads to the elimination of lipid hydroperoxide from membranes. Heightened lipid peroxidation extends to collagen oxidation, inducing alterations, fragmentation, aggregation, and conformational changes, affecting tissue function and integrity.[18,45] This highlights the link among PCP exposure, lipid peroxidation, and subsequent alterations in cellular and tissue integrity.

Altered Energy and Amino Acid Metabolism as Revealed by Serum Metabolomics. Serum metabolomics research indicated 60 days of PCP exposure in catfish *H. fossilis* resulted in significant metabolic alterations. These alterations primarily point to oxidative stress, energy metabolism dysregulation, reproductive system anomalies, and neurotransmitter imbalances. Under normal circumstances, the primary energy source is the oxidation of glucose (Gls) through aerobic respiration. In hypoxic environments, the primary energy source switches to anoxic respiration, which produces energy less efficiently than aerobic respiration.[46] Aerobic respiration, involving the oxidation of glucose, typically serves as the primary energy source. The mitochondrial reactions of the TCA cycle produce a small amount of ATP and reducing equivalents (NADH and $FADH_2$), enabling the continuation of the respiratory process. Acetyl-CoA and pyruvate are converted to citrate, which is then interconverted into several organic acids, including ketoglutarate, succinate, fumarate, and oxaloacetate. Through the mitochondrial electron transport chain, the oxidative phosphorylation of NADH and $FADH_2$ produces a significant amount of ATP.[1,47,48] According to the metabolomics profiling, PCP exposure in catfish resulted in a significant decrease in glucose, pyruvate, lactate, citrate, and glutamate; these differential metabolites are linked to energy metabolism. Significantly lower glucose levels indicated a higher rate of glycolysis. The end product of glycolysis is pyruvate, which, under hypoxic conditions, is converted to lactate by lactate dehydrogenase (LDH).[1,49]

Since pyruvate is essential for pyruvate metabolism and the tricarboxylic acid (TCA) cycle, the lower lactate levels suggest that pyruvate breakdown has been disrupted or LDH activity is inhibited.[50,14] The decreased citrate was an indicator of a TCA cycle that may have been impeded by mitochondrial injury and the resulting alteration in the ATP supply. Hence, exposure to PCP caused damage to mitochondria, indicating that acetyl-CoA metabolism was disturbed, which may have affected the TCA cycle. Decline in acetyl-CoA results in a decrease of 3-HB. The TCA cycle and pyruvate metabolism are both important steps in energy metabolism.[50−52] Since pyruvate is essential for pyruvate metabolism and the TCA cycle (Figure 9). The decreased lactate level reflected its excessive consumption to replenish insufficient energy production.[53] Hence, exposure to PCP caused damage to mitochondria, indicating that acetyl-CoA metabolism was disturbed, which may have affected the TCA cycle and consequently led to the decline in Glu levels. The decrease in energy metabolites observed in the current study was caused by disturbance in energy metabolism following PCP treatment for 60 days to *H. fossilis*.

AAs are the best fatty acid synthesis precursors in fish. In the mitochondria, Glu is converted to acetyl-CoA.[54] This acetyl-CoA then leaves the mitochondria by forming citrate in the cytosol, which is then broken down by ATP-dependent citrate lyase into acetyl-CoA and oxaloacetate.[55] The fatty acid synthase complex uses acetyl-CoA to synthesize long-chain fatty acids in the cytosol. Fish may preferentially employ the circulating Glu for the production of lipids rather than glucose.[56] In juvenile gilthead seabream (*Sparus aurata*), Glu stimulates glycolysis, glycogenesis, and lipogenesis to improve glucose utilization in the liver.[57] In aquatic organisms, the equilibrium of lipids and glucose may be significantly regulated by Glu. The body can make Ala, a nonessential amino acid, by converting pyruvate. One of the most significant amino acids generated by muscle, it is highly concentrated in muscle and serves as a significant energy source. Alanine is an inhibitory neurotransmitter in the brain, just like γ-aminobutyric acid (GABA) and taurine.[53,58] The serum Alanine level fell in the current investigation, possibly as a result of the ability of

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Alanine to operate as a glycogenetic amino acid to restore deficient energy sources.

Histidine (His), valine (Val), leucine (Leu), Ala, Glu, and Gly were all significantly altered by PCP exposure for 60 days to *H. fossilis*, according to the ^{1}H NMR metabolomics investigation. Moreover, as branched-chain amino acids (BCAAs), Val and Leu are recognized to be necessary precursors for a number of metabolic processes in an organism, including protein synthesis, the production of energy, and growth development.[50,59,60] A rise in protein synthesis to restore or repair disrupted membrane protein structure is suggested by decreasing BCAA levels[14,61,53,62] and BCAAs are also crucial for controlling the process of protein turnover. ROS could damage and fragment proteins by attacking them.[14] After exposure to PCP, a significant drop in Val and Leu was seen in catfish *H. fossilis* serum, and from this we could infer that it may be because they were being used more frequently to synthesize and repair the damaged proteins. In response to circulating catecholamines, Ala can modify the peripheral nervous system and cause a cardiac response.[50,62] In order to regulate the histaminergic system, neurohormones and neurotransmitters need the His as the major component.[50,63] Comprehensively,[59] also explained that the zebrafish embryo produced reaction to environmental chemicals resistance and hypothesized that the decreased Ala and His may have influenced the spontaneous movements of the heartbeat. These striking results imply that difenoconazole exposure may have perturbed amino acid metabolism, which in turn affected the spontaneous movements and growth development and this was consistent with the study[64] on HDC exposure-lacking female mice. His affects fish cataract prevention, buffering, and muscle growth.[65−68] These striking results imply that PCP exposure may have perturbed Ala which in turn may affect spontaneous movement and growth development that may lead to behavioral abnormalities and inhibit histidine from serving different functions in catfish *H. fossilis* like growth, buffering, and cataract prevention. The amino acid Gly is one of the nonessential amino acids, but it also plays a number of important physiological roles, including growth performance, feed efficiency, white blood cell (WBC) blood-neutrophil and monocyte quantity and percent, poor lysozyme synthesis, skin mucosal immunity, and increasing GSH while lowering GR.[69−72] Arginine (Arg) is necessary for the synthesis of creatine. The amidino group of Arg was transferred to Gly by Arg: Gly amidinotransferase,[73] creating guanidinoacetate, the direct precursor to creatine. For the storage and buffering of high phosphate-bound energy in brain and skeletal muscle with high energy demand, this shuttle system proved crucial.[74] The significant drop in glycine found in the serum of PCP-intoxicated catfish may indicate a change in the creatine/phosphocreatine shuttle system function as well as reduced growth performance, feed efficiency, white blood cell (WBC) blood-neutrophil and monocyte quantity and percent, poor lysozyme synthesis, skin mucosal immunity, and GSH while increasing GR. Fish erythrocytes are susceptible to oxidative circumstances (lower GSH, higher GPx, and higher LPO), which may have a tendency to cause hemolysis.

PCP Induced Neurotoxicity and Abused Reproductive Health. Pyridoxal phosphate, the active form of vitamin B6, is used as a cofactor by glutamic acid decarboxylase (GAD) in neurons to synthesize γ-aminobutyric acid (GABA) from glutamate (Glu). The channel catfish and goldfish brains both contain GAD (two isoforms, GAD65 and GAD67).[75,76] GABA is the main inhibitory neurotransmitter in the central nervous system and is involved in the regulation of excitatory responses, sex steroid regulation, anoxic metabolic depression, and pituitary hormone release.[77] In fish, the pituitary gland releases growth hormone, a process in which both Glu and GABA play a crucial role.[78] Glu, being the principal excitatory neurotransmitter in the central nervous system of vertebrates, acts as a hypophysiotropic regulator. This means it controls the release of various hormones, including growth hormone, luteinizing hormone, prolactin, and possibly melanocyte-stimulating hormone or somatolactin, a protein produced by the pituitary gland.[79] Furthermore, Glu and GABA stimulate feed intake by inducing the production of peptide and gaseous neurotransmitters from orexigenic neurons in the hypothalamus.[55] In the current work, we observed a considerable reduction in Glu, which may have an impact on the brain synthesis of many hormones and result in several abnormalities in catfish.

According to some research, the Glu increased sperm motility and the rate at which it fertilized rainbow trout eggs.[80] The addition of Glu boosted steroidogenesis in rainbow trout ovarian follicles.[81] Fish luteinizing hormone is released when Glu and its metabolite GABA are present.[78] In the present research, we see a rapid decrease in Glu levels that may have an effect on the production and action of many hormones involved in gonad development as well as steroidogenesis, ultimately causing a decline in reproductive activity.

In the current investigation, female *H. fossilis* (His, Val, Glu, Leu, Ala, 3-HB, pyruvate, citrate, and lactate) exposed to PCP for 60 days had more amino acid altered than males *H. fossilis* (Val, Leu, Gly, and Gls), affecting several pathways, including amino acid metabolism, energy metabolism, oxidative stress, neurotoxicity, and reproduction, whereas in males, it was energy metabolism and amino acid metabolism. Therefore, we might draw the conclusion that in our experiment, females are relatively more prone than males to experience a toxicological effect of chronic PCP exposure.

CONCLUDING REMARKS

Despite regulatory prohibitions in many countries, the persistence of PCP in the environment is a persistent challenge. Its continued identification in various environmental compartments, including water bodies and sediments, indicates ongoing contamination and underscores the need for sustained efforts in monitoring, regulation, and remediation. The resilience of PCP in the environment necessitates comprehensive strategies to address its persistence and mitigate its impact on aquatic ecosystems and the organisms inhabiting them. To the best of our knowledge, this is the first study to examine the toxic effects of PCP in catfish *H. fossilis* using a comprehensive "omics" method. The study exquisitely demonstrated the effectiveness of metabolomics in understanding the mechanisms of PCP toxicity and its potential application in risk assessment in toxicology research. First, the study demonstrated that chronic exposure to PCP harms catfish *H. fossilis*, through increasing oxidative stress, as seen through high levels of lipid oxidation and hydrogen peroxide along with decreased antioxidant enzyme activity (crucial to defend against oxidative stress and activate membrane repair mechanisms). This stress is worsened by a weakened antioxidant defense system, making tissues vulnerable to damage from harmful metabolic products produced by PCP exposure. PCP exposure also causes alterations in serum

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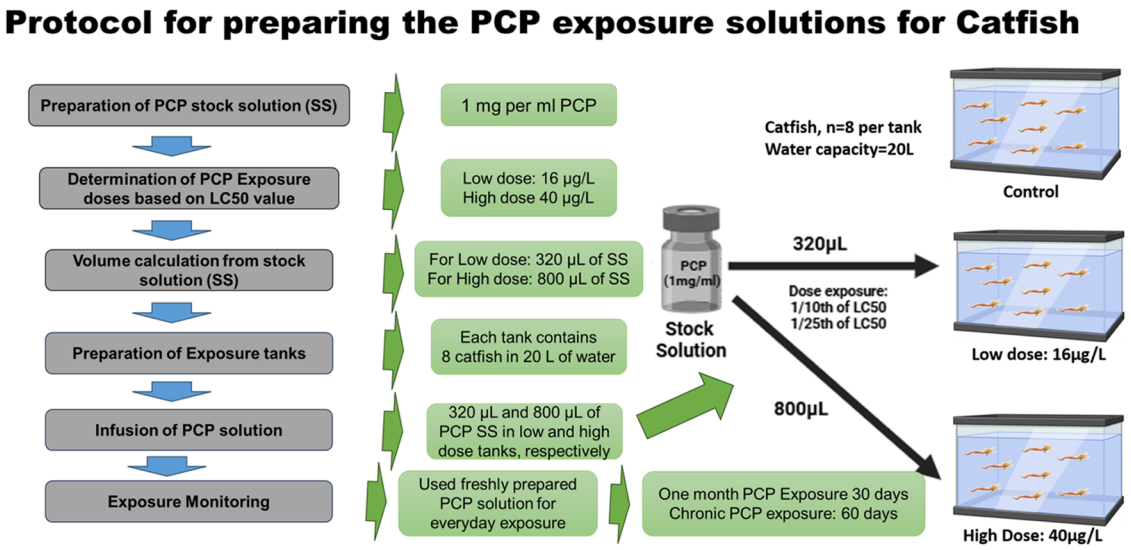

Figure 10. Schematic showing study protocol used for investigating the intervention effect of pentachlorophenol (PCP) on a preclinical catfish model.

metabolites and damage to catfish liver and gonad tissues causing histopathological alterations such as leukocyte infiltration, hepatocyte degeneration, and abnormal sperm distribution in PCP-exposed catfish. Histological aberrations match the severity of PCP exposure and metabolomics analysis revealed dose-dependent changes in serum metabolites. Metabolomics also found differential metabolic changes in the sera of male and female catfish due to PCP exposure. Specifically, PCP exposure led to increased levels of amino acids such as alanine in low-dose exposed female catfish, while histidine, valine, glutamate, leucine, pyruvate, 3-hydroxybutyrate (3-HB), citrate, and lactate levels were decreased. In male catfish, decreased levels of valine, leucine, glucose, and glycine were observed. These changes suggest that PCP exposure affects various metabolic pathways, including oxidative stress, energy metabolism, amino acid metabolism, neurotransmitter balance, hormonal balance, membrane stability, degradation, and repair activity in catfish. These findings will contribute to the knowledge of the toxicological effects of PCP on aquatic organisms and underscore the importance of monitoring and regulating environmental pollutants to protect aquatic animals and improve human health.

MATERIALS AND METHODS

The histopathological features of tissues were characterized following the methods described previously.[81] As the present study is an extension of the previous study reported from our lab,[82] these experiments are performed following the methodological details as described previously,[82] including ethical approval, fish collection and acclimatization, and histopathological examination of liver and gonad tissues. Additionally, the present study also involved the comparative evaluation of antioxidant status and nonenzymatic parameters of oxidative stress as described previously (explicit details are also included as Annexure-I in the ESM).[83−89]

Preparation of PCP Exposure Solution. The protocol for preparing pentachlorophenol (PCP) exposure solutions and administering exposure under controlled conditions for three catfish groups: control, low dose (16 μg/L), and high dose (40 μg/L) is schematically shown in Figure 10. Briefly, the PCP stock solution (SS) is prepared at a concentration of 1 mg/mL. The previously reported LC50 (lethal concentration for 50% of the population) values were used to decide the PCP exposure doses: low dose: 16 μg/L (1/25th of LC50) and high dose: 40 μg/L (1/10th of LC50). For each study group, a 20 L water tank containing eight catfish was used and infused with appropriate volumes of PCP stock solution, i.e., 320 and 800 μL of SS, respectively, for low- and high-dose 20 L water tank (Figure 10). Fresh PCP solution is prepared daily for consistent exposure to 30 and 60 days. The purity of the PCP exposure was confirmed using mass spectrometry (MS) and 1D 1H and 13C NMR analysis. The results are shown in the ESM (see Figures S3 and S4).

Procedure for Serum Collection and NMR Sample Preparation. After 60 days of PCP exposure, the blood was extracted from catfish via caudal puncture, collected into microcentrifuge tubes, and allowed to coagulate at room temperature for 3−4 h. The samples were then centrifuged at 4200 rpm for 15 min at 4 °C. The resulting supernatant (serum) was separated and stored at −80 °C in an ultradeep freezer until further analysis. Prior to NMR experiments, the stored serum samples were retrieved from the −80 °C ultrafreezer and kept at room temperature for thawing. Each serum sample (300 μL) was mixed with 300 μL of 50 mM sodium phosphate buffer (prepared in 100% D_2O solvent, pH 7.4 and 0.9% saline) and centrifuged at 16,278g for 5 min. Subsequently, the supernatant from each sample (550 μL in volume) was transferred to 5 mm NMR tubes (from Norrell, USA; https://secure.nmrtubes.com) for NMR data collection. Deuterium oxide (D-99%) and d_6-DSS were sourced from Sigma-Aldrich (St. Louis, MI, USA).

NMR Measurements. 1D ^{1}H NMR experiments were performed at 300 K using an 800 MHz NMR spectrometer (Bruker Avance-III, Bruker BioSpin GmbH, Germany) equipped with an actively shielded z-gradient system and a TCI Cryoprobe. In order to surmount the NMR signals of metabolites and suppress broad signals of protein and other macromolecules, the standard Carr−Purcell−Meiboom−Gill (CPMG) spin−echo pulse sequence (present in Bruker library with name CPMGPR1D with water presaturation block and allowing $T2$ filtering as well).[90,91] The broad NMR signals of protein and other macromolecules were suppressed using $T2$

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filtering time (i.e., $[\tau-180^\circ-\tau]_n$) approximately equal to 80 ms. This was achieved by using spin echo time (2τ) equal to 600 μs, loop counter (n) equal to 128, whereas 180° radio frequency (RF) pulse length was set to 25 μs. Additional acquisition parameters included a relaxation delay of 5 s, ^{1}H spectral width of 20 ppm, number of scans of 128, and size of free induction decay (FID) equal to 64 K data points. The acquisition time for each CPMG NMR experiment was ∼15 min. Each spectrum was then manually corrected for phase and aberrant baseline distortions and then calibrated for Lactate methyl group signal at 1.3102 ppm using Bruker software TopSpin (v3.6.1).

The spectrum was then opened in the PROCESSOR module of NMR Suite of commercial software CHENOMX (v8.6, www.chenomx.com) and baseline was further refined for improved quantitative profiling.[92] All of the NMR spectra for control and PCP-exposed catfish study groups were then binned (with bin size equal to 0.04 ppm), and each bin was normalized with respect to the total spectral intensity. The resulting data matrix was imported into an Excel sheet and saved in the "CSV" file format after adding the class information according to the study groups.

Multivariate Statistical Analysis. NMR-based serum metabolic profiles of PCP-exposed and normal control groups of catfish were compared using multivariate statistical analysis tools such as PCA (an unsupervised approach) and PLS-DA (supervised approach).[93,94] For this, the multivariate data matrix was used as an input in the MetaboAnalyst web-based analysis software (v5.0, http://www.metaboanalyst.ca/).[93,94] Multivariate analysis was performed separately for two data matrices of normalized spectral features (representing serum metabolite concentration profiles) following the steps described previously.[95,96] One data matrix contained metabolite concentration profiles for female catfish, and another data matrix contained metabolite concentration profiles for male catfish *H. fossilis*.

PCA was employed to identify outliers within the data set and intrinsic clustering of samples within the study groups, followed by PLS-DA to enhance class discrimination.[97] As PLS-DA model may overfit the data, therefore, the classification accuracy for top 5 latent variables of PLS-DA model was assessed using a 10-fold cross-validation algorithm.[93,97] Permutation analysis was conducted 100 times to cross-validate the PLS-DA models, and the quality of the PLS-DA models was assessed based on cross-validation parameters $R2$ (explained variance, so goodness-of-fit) and $Q2$ (indicating the model's predictive capability).

The PLS-DA model also provided information about the variable importance in projection (VIP) scores, which were used to identify the metabolites responsible for discrimination between study groups. Before considering the discriminatory metabolites (metabolic features) for biological interpretation, these features were also tested for statistically significant differences between the study groups using the Mann–Whitney U test, which accounts for unequal group variance. The resulting p-values were adjusted to minimize the false discovery rate (FDR). MetaboAnalyst (v5.0) was used to perform metabolic pathway analysis, integrating pathway enrichment, and topology analyses. Key metabolites altered in PCP-exposed male and female catfish compared with normal catfish were used as inputs for this analysis.

■ ASSOCIATED CONTENT

Data Availability Statement

The manuscript and its supporting information contain all of the relevant data. The raw NMR spectral data will be available upon request to the corresponding authors.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03407.

> Methodology used for evaluating antioxidant status and nonenzymatic parameters of oxidative stress in study samples (Annexure-I); summary of previous studies reporting PCP contamination in groundwater and other water bodies (Table S1); results derived from partial least square-discriminatory analysis (PLS-DA) model showing intervention effect of PCP-treated (for 30 days) female and male *H. fossilis* in comparison to control *H. fossilis* (Figure S1); variable importance in projection (VIP) score plots derived from partial least square-discriminatory analysis (PLS-DA) model analysis showing metabolic changes in PCP-treated male and female catfish, *H. fossilis* for 60 days and 30 days (Figure S2); mass spectrometry (MS) analysis of exposure solutions prepared containing different concentrations of pentachlorophenol (PCP) (Figure S3); and NMR analysis of solution sample containing 1 mg/mL pentachlorophenol (PCP) in 100% deuterated dimethyl sulfoxide (DMSO-d_6) (Figure S4) (PDF)

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Author Contributions

D.K. and R.C.: conceptualization of idea and study design. R.C. and S.S.: experiments on catfish, data analysis, and generating the results. S.S. and S.Y.: preparation of serum samples for NMR-based metabolomics, NMR data collection. D.K. and S.Y.: metabolomics data analysis. S.S. and D.K.: manuscript drafting manuscript preparation.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

PCPpentachlorophenol
H. fossilisHeteropneustes fossilis
NMRnuclear magnetic resonance
CPMGCarr−Purcell−Meiboom−Gill
1D/2Done/two-dimensional
EPAEnvironmental Protection Agency
ESMelectronic Supporting Information
NCnormal control
PCAprincipal component analysis
PLS-DAprojection to least-squares discriminant analysis
TCHQtetrachlorohydroquinone
TCBQtetrachloro-*p*-benzoquinone
NADHnicotinamide adenine dinucleotide + H
GSHglutathione (reduced)
LPOlipid peroxidation
H_2O_2hydrogen peroxide
CATcatalase
SODsuperoxide dismutase
GPxglutathione peroxidase
3HB3-hydroxybutyrate
GPCglycerophosphocholine
ROSreactive oxygen species
kDakilo dalton
GSIgonadosomatic index
HSIhepatosomatic index
ANOVAanalysis of variance
SEMstandard error in mean
Zrpzona radiata proteins
Vtgvitellogenin
HDLhigh-density lipoproteins
LDLlow-density lipoproteins
VLDLvery low-density lipoproteins
μgmicrogram
DMSdimethyl sulfone
BCAAbranched-chain amino acid
Gluglutamate
Glsglucose
Glnglutamine
NAG*N*-acetyl glycoprotein
Phephenylalanine
OACO-acetyl carnitine
ICTinterlobular connective tissue

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