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Ecological Toxicity of Pesticides, Heavy Metals and Microbial Pollution, in the Running Surface Water of Jhal Magsi, Pakistan

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Regular monitoring of water quality is essential for providing clean drinking water and protection of aquatic environments. The aim of the current study is to evaluate the quality and effects of low biodegradability, accumulation of heavy metals, and organic compounds on the running water sources of the district of Jhal Magsi. The concentrations of herbicides (DDT (2,4 D) and Cyanazine) and insecticides (chlorpyrifos, Aldrin, and Dieldrin) were determined by gas chromatography and the concentrations of heavy metals (Pb and Cd) and trace elements (Zn and Mn) were detected by anatomic absorption spectrophotometer. *E. coli* and *P. aeruginosa* were identified by Gram staining and biochemical tests. DDT (2,4 D), Cyanazine, Chlorpyrifos and Aldrin & Dieldrin were detected at 0.9±0.058, 0.08±0.006, 1.01±0.577 and 1.1±0.577 mg L^{-1} respectively; these values were higher than WHO safety limits. The heavy metals Pb and Cd were detected in all the samples and the highest range for Pb was 2.03±0.577 mg L^{-1}, while that for Cd was 0.48±0.058 mg L^{-1}. Trace elements Zn and Mn concentrations were 0.305±0.058 and 0.99±0.058 mg L^{-1}, respectively. *E. coli* and *P. aeruginosa* were identified with 51.51 and 48.48%, respectively. The presence of higher concentrations of pesticides and heavy metals and their synergistic effect indicate a risk for both human health and aquatic organisms. Therefore, continuous monitoring of heavy metals and pesticides is necessary in the water reservoir of Jhal Magsi to ensure drinking safety. Bacterial assessment can aid in accepting bioremediation processes in water.
Keyword: Synergistic toxicity, environmental monitoring, agnostic relationship, gas chromatograph, atomic absorption spectroscopy

INTRODUCTION

Surface water, like lakes and rivers, is used for multiple purposes, supplying water for agricultural irrigation, drinking water, energy production and fisheries. However, these important resources are endangered by fast-rising populations, domestic, and agricultural pollution, and developing technologies (Akanwa, 2021). The water pole is polluted by agricultural, sewage, and industrial waste. Metals and Pesticides contaminate surface water by runoff waste. Environmental pollutants enter into groundwater when rainwater parasites dissolve pollutants in the soil. Recurrent principles like wineries often use trace elements, such as Cu or Zn. heavy metals and Pesticides accumulate in agricultural soils and finally spread to surface and ground water after rainfall (Madhav *et al*., 2020). Heavy metals can enter naturally, but maximum contaminants come from excess untreated wastewater discharge and spills, soil, and dumping of human activities. Because of their persistent and non-degradable nature, pollution in water environments is an important problem that affects environments, human health, and animals (Saravanan *et al.*, 2022). Predicting the transportation of pollutants from soils to aquatic environments is important for risks and assesses of toxic level. The president's use of organochlorine pesticides an important health fears. They can easily enter the cell membranes because of their lipophilic nature. Anthropogenic activities release heavy metals into the environment because of a possible risk of contrary health impacts on both non-target wildlife and humans (Kishor *et al.*, 2021).
The toxicity of a component can change its effect on other components, causing unexpected health effects. This has

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posed an important challenge for ecotoxicology and environmental health in recent decades. Many studies in the literature have evaluated how different chemical mixtures interact and combined (synergistic or antagonistic, additive) toxicity occurs (Liu and Sayes, 2024). In addition, numerous studies have been conducted to evaluate the acute toxicity of multicomponent chemical mixtures (Martin *et al.*, 2021). Antagonistic or synergistic relationships occur when the toxicity of a mixture is less or greater than the sum of the toxicities of its components, respectively. In addition to these conflicting results, regulatory authorities typically impose regulations that treat acceptable concentrations of pollutants as independent, even when they are present in mixtures (Nikos *et al.*, 2024).

When water holds fecal material from industry, cattle farms, and hospitals, they contain loaded bacteria. *Escherichia coli* are known as a common indicator of fecal contamination. The bacteriological investigation of water contaminated by sewage shows that it is unsafe for recreational or drinking purposes (some *et al.*, 2021). Microbes can help to clean wastewater. They feed organic matter present in sewage and use the nutrients for growth, which in turn enhances the cleaning process of the wastewater. This action progresses the quality of water and its self-cleansing ability. Additionally, it also helps to decrease the biochemical oxygen demand (BOD) and removes unpleasant odors (Brix, 2020).

For bioremediation, it is necessary that the environmental conditions are suitable for the growth and activity of these microorganisms. In not ideal conditions, adjustments are made to the environmental factors to make it more favorable for the growth of these microorganisms and therefore, speed up the degradation procedure. It is important to note that the microorganisms used in bioremediation are present in the environment, which stops any damage to the environment (Thacharodi *et al.*, 2024). In this study, we provide an overview of the pollution status of two major pollutants; pesticides and heavy metals and how their synergetic effect can affect the environment. Additionally, we explore the prevalence of pathogen contamination in freshwater bodies in the Jhal Magsi district.

MATERIALS AND METHODS

A total of 75 Water samples were collected from different points of raining water resources (natural spring, canals and river water) of district Jhal Magsi Balochistan Pakistan (Figure 1).

***Quality Assurance and Preservation of Samples*:** The 1000 mL water samples were divided into three groups for different parameters detection. 100 mL for bacterial analysis of each sample was taken in separate labeled bottles. The remaining 500 mL for pesticides, sampling bottles were cleaned once with 30 mL acetone and 400 mL samples were preserved by adding 5 mL of HNO_3 for heavy metals analysis. Samples were brought to the laboratory for analysis of bacteria within 24 hours.

Figure 1. Showing different sites River Mula, Sukleji, Gandawah, Shoran, Mirpur jamot, and Fathe Pur where water samples were collected from the district Jhal Magsi

***Pesticides analysis*:** Water samples were extracted using a separatory funnel with 30 mL of dichloromethane solvent. The funnel was shaken for 5 minutes with periodic venting. After 15 minutes, the organic layer was separated from the water phase. The extract of dichloromethane was together in a 250 mL Erlenmeyer flask. This extraction procedure was repeated three times, combining the extracts in the flask. The flask was then rotated at 200 rpm for one hour using a rotary evaporator. Removal and purification of interferences were removed by using silica gel columns. Extracts were evaporated and reconstituted with 0.5 mL of hexane for analysis. Quality control blanks of 1 liter double distilled water showed no pesticides. Column temperature was programmed from 80 to 310 ºC with specific time intervals. Straight injection was used with injector maintained temperature at 225 ºC (Ciornea *et al.*, 2016). The quantitative analyses of the selected pesticides were performed by gas chromatography, as shown in figure 2.

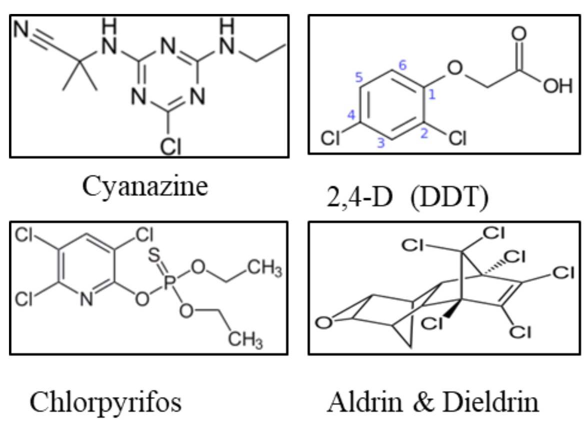

Figure 2. The pesticides in the surface water of the district of Jhal Magsi

***Heavy metals*:** Water samples were digested for heavy metal detection following the Fong SS method.

***Preparation of standard reagents*:** Standard solutions for each metal (Pb, Cd, Zn, and Mn) were prepared with different concentrations up to 1000 ppm (Fisher Chemicals, U.K) by:

$$\mathrm{Vs(mg\ L)} = \frac{\mathrm{Cw} \times \mathrm{Vd}}{\mathrm{Cs}}$$

V_s = volume (mL) primary standard (stock) add with diluent to prepare diluted working standard; C_w = Required concentration of working standard (mgL^{-1}); V_d = Desire final volume; C_s = Primary standard concentration of Stock solution

For the analytical determination, ultrapure compounds were used. Deionized water was used for distillation, after which the mixture was stored in glass containers (Mustafa *et al.*, 2017).

***Bacterial Analysis*:** Water samples collected in sterile bottles were processed for identification of bacterial species. 100 mL of water sample were put in to 10 mL of broth (Luria Bertani). These falcon tubes were incubated at 37 °C for cultivation 24 hours. Next day, grown bacterial suspensions were cultivated on nutrient agar and then incubate at 37 ºC till next day. Microscopic examination was done by gram staining and biochemical identification was performed by different tests for identification of *E. coli* and *P. aeruginosa* (Malik *et al.*, 2023).

***Statistical Data Analysis*:** All data for pesticides and heavy metals were presented as the average ± standard error. The data were analyzed according to the central limit theorem. Therefore, Pearson's correlation was used.

RESULTS

The deducted pesticides (herbicide and insecticides) from running surface water of district Jhal Magsi was DDT (2,4 D), Cyanazine, Chlorpyrifos and Aldrin & Dieldrin. The highest deducted ranges from the normal ranges of WHO were 0.9±0.058, 0.08±0.006, 1.01±0.577 and 1.1±0.577 mg L^{-1} for DDT (2,4 D), Cyanazine, Chlorpyrifos and Aldrin & Dieldrin respectively shown in Table 1.

Heavy metal Pb, Cd, Zn and Mn were analyzed from the surface running water of district Jhal Magsi. The Pb and Cd were deducted in all the samples of district, the highest ranged of Pb, Cd Zn and Mn were 2.03±0.577, 0.48±0.058, 0.305±0.058 and 0.99±0.058 mg L^{-1} respectively (Table 2).

E. coli and *P. aeruginosa* were identified from samples of selected sites. The detected *E. coli* and *P. aeruginosa* were shown 51.51 and 48.48% respectively. In sample site of Gandawah both bacteria were identified in the same ratio 4/5 (Table 3). The correlation was analyzed shown in table 4. According to the results of correlation analysis, Pb has shown inverse relationship with Cd, DDT (2,4 D), Aldrin & Dieldrin, *E. coli, P. aeruginosa;* Cd show a correlation with Chlorpyrifos, DDT (2,4 D), Aldrin & Dieldrin; Zn has a strong correlation with Mn and Chlorpyrifos; DDT (2,4 D) has a correlation with Chlorpyrifos; Chlorpyrifos has a correlation with Aldrin & Dieldrin ($p<0.01$). Heavy metals and pesticides were shown Combine synergistic, antagonistic and additive effects (Table 5).

Table 1. Pesticides in Surface water samples from district Jhal Magsi.

Sr.	Sites	DDT (2,4 D)	Cyanazine	Chlorpyrifos	Aldrin & Dieldrin
				mg L^{-1}	
Types of Pesticides		Herbicide	Herbicide	Insecticide	Insecticide
Total no samples		54	52	49	31
WHO limits (MRL)		0.03	0.0006	0.03	0.00003
1	Peer chhatta	0.21±0.058	0.02±0.006	0.04±0.006	0.87±0.058
2	kotra	0.43±0.058	0.04±0.006	0.21±0.058	0.01±0.001
3	Gandawah	0.04±0.006	0.06±0.006	0.45±0.058	0.76±0.058
4	Gajan	0.01±0.001	0.05±0.006	0.09±0.006	0.21±0.058
5	Shoran	0.81±0.058	0.04±0.006	0.39±0.058	0.09±0.006
6	Mir Pur	0.90±0.058	0.02±0.006	0.68±0.058	0.93±0.006
7	Patri	0.31±0.058	0.01±0.001	0.91±0.006	0.34v0.058
8	Kot Magsi	0.62±0.006	0.03±0.006	0.80±0.058	0.72±0.058
9	Mirpur jamot	0.51±0.058	0.05±0.006	0.04±0.006	0.21±0.058
10	sukleji	0.33±0.058	0.08±0.006	0.71±0.058	0.40±0.058
11	Gahela	0.21±0.058	0.06±0.006	0.04±0.006	0.52±0.058
12	Farom	0.03±0.006	0.04±0.006	0.31±0.058	0.43±0.058
13	Fathe Pur	0.53±0.058	0.03±0.006	1.01±0.577	0.62±0.058
14	Khari	0.49±0.058	0.06±0.006	0.68±0.058	1.10±0.577
15	River Mula	0.31±0.058	0.04±0.006	0.45±0.058	0.67±0.058

*All selected pesticides were taken (Average ± SE (standard error)) in mile gram per litter (mg L^{-1}) in all water samples. Maximum residue limit (MRL) in drinking water for various pesticides were taken (WHO, 2017)

Table 2. Heavy metal in surface water of district Jhal Magsi

Sr.	Name of area	Pb	Cd	Zn	Mn
		mg L^{-1}			
Type of metals		Heavy metal	Heavy metal	Trace metal	Trace metal
Total present metals		67	64	66	59
EPA/ WHO (MRL)		<0.05/0.01	0.01/0.003	5/3	<0.5/0.5
1	Peer chhatta	1.72±0.577	0.090±0.006	0.088±0.006	0.160±0.058
2	kotra	1.64±0.577	0.105±0.058	0.083±0.006	0.101±0.058
3	Gandawah	1.97±0.577	0.104±0.058	0.061±0.006	0.110±0.058
4	Gajan	2.03±0.577	0.110±0.058	0.058±0.006	0.104±0.058
5	Shoran	1.98±0.577	0.210±0.058	0.088±0.010	0.270±0.058
6	Mir Pur	1.87±0.577	0.340±0.058	0.101±0.058	0.120±0.058
7	Patri	1.99±0.577	0.100±0.001	0.305±0.058	0.990±0.058
8	Kot Magsi	1.78±0.577	0.480±0.058	0.099±0.006	0.440±0.064
9	Mirpur jamot	1.88±0.577	0.101±0.058	0.097±0.001	0.340±0.058
10	sukleji	1.96±0.577	0.104±0.058	0.088±0.006	0.140±0.058
11	Gahela	1.99±0.577	0.102±0.058	0.078±0.006	0.130±0.058
12	Farom	1.98±0.577	0.103±0.058	0.098±0.001	0.870±0.058
13	Fathe Pur	1.88±0.577	0.110±0.058	0.102±0.058	0.340±0.058
14	Khari	1.78±0.577	0.103±0.058	0.205±0.058	0.370±0.058
15	River Mula	1.86±0.577	0.099±0.006	0.169±0.058	0.280±0.058

*Pb (Lead), Cd (Cadmium), Zn (Zinc) and Mn (manganese). All the heavy metals vales were taken (Average ± SE (standard error) in milligram per litter (mg L^{-1}) in all water samples. Maximum residue limit (MRL) in drinking water for various heavy metals were taken (WHO, 2021)

Table 3. Total number and percentage of Bacteria

S .no	Name of area	*E. coli*	*P. aeruginosa*
1	Peer chhatta	3/5	3/5
2	kotra	2/5	2/5
3	Gandawah	4/5	4/5
4	Gajan	3/5	3/5
5	Shoran	1/5	1/5
6	Mir Pur	1/5	1/5
7	Patri	2/5	2/5
8	Kot Magsi	3/5	3/5
9	Mirpur jamot	3/5	3/5
10	sukleji	1/5	1/5
11	Gahela	1/5	1/5
12	Farom	2/5	2/5
13	Fathe Pur	2/5	2/5
14	Khari	1/5	1/5
15	River Mula	3/5	3/5
Total no of Bacteria		34/75	32/75
Percentage of Bacteria		51.51%	48.48%

*From every site five (5) water samples were collected for bacterial analysis and a total of 75 water were used for bacterial detection

DISCUSSION

The investigations carried out on the running surface water of district Jhal Magsi. Different pesticides and fertilizers used for the proper growth of plants. Exposure to heavy metals and pesticides has caused a high number of cancer cases. A current risk assessment, which also took from animals, estimated that exposure to dioxin-like and dioxin compounds (such as PCBs) could result in a cancer up to 10^{-4} per year.

In the present study, many pesticides were identified by using chromatographic experiments with a standard solution. The same technique was utilized by Wang *et al.* (2007) and Nguyen *et al.* (2019) for the quantitation and determination of pesticides in surface water through gas chromatography–mass spectrometry. Júnior and Re-Poppi (2007); Cortada *et al.* (2009), were conduct research on various pesticides including Aldrin and Dieldrin have been detected in water samples. These pesticides were found in river, surface, and tap water, with higher concentrations observed in surface water samples. Chlorpyrifos organophosphorus pesticides were found higher in an aqueous environment (Tiwari and Guha, 2013). Arain *et al.* (2018) studied the chlorpyrifos concentration in the surface and ground water of Okra. These results are consistent with the findings of the present study. Chlorpyrifos was detected in all the water samples from the district.

Cyanazine was detected in surface water samples of district Jhal Magsi; as Kone *et al.* (2024) detected cyanazine from the surface water. Cyanazine has Acute toxicity is moderately toxic to mammals. Furthermore, the herbicide DDT was also detected in the water of district Jhall Magsi (Strong *et al.*, 2015; Burgos-Aceves *et al.*, 2021). The contact of DDT can cause contrary effects on the reproduction and liver. High doses of DDT can cause seizures, vomiting and tremors in humans.

In the present study Cd and Pb was deducted in higher concentration and Zn and Mn were deducted in majority of

Table 4. Pearson correlation chart for Heavy metals, Pesticides and Bacterial analysis

	Pb	Cd	Zn	Mn	DDT (2,4 D)	Cyanazine	Chlorpyrifos	Aldrin & Dieldrin	*E. coli*	*P. aeruginosa*
Pb	1.000									
Cd	-0.241	1.000								
Zn	0.011	-0.102	1.000							
Mn	0.188	0.016	0.677**	1.000						
DDT (2,4 D)	-0.307	0.493*	0.107	-0.178	1.000					
Cyanazine	0.256	-0.321	-0.383	-0.414	-0.304	1.000				
Chlorpyrifos	0.026	0.371*	0.503**	0.374	0.400*	-0.235	1.000			
Aldrin & Dieldrin	-0.243	0.335*	0.155	-0.015	0.084	-0.090	0.333*	1.000		
E. coli	-0.091	0.041	-0.187	0.019	-0.474	-0.132	-0.237	0.025	1.000	
P. aeruginosa	-0.091	0.041	-0.187	0.019	-0.474	-0.132	-0.237	0.025	1.000	1.000

*Pb (Lead), Cd (Cadmium), Zn (Zinc) and Mn (manganese). ** For Pearson Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 levels (2-tailed) and –ive show inverse relationship.

Table 5. Relationship between exposures of Heavy metals, Pesticides and their combine Effects

S.no.	Metals	Pesticides	Effects	References
1	Cd,	Chlorpyrifos	Cause damage and Oxidative stress to the mitochondria in neuronal cells.	Xu *et al*. (2017)
2	Zn	Chlorpyrifos	Cause cellular stress responses and reproduction problems.	García-Gómez *et al*. (2019)
3	Ni, Mn, Pb, Cd, Zn	Different pesticides, PCB and chemical	Combine effect the heavy metals residual attractive forces and Unbalanced forces (adsorption mechanism).	Yang *et al*. (2020)
4	Cd, Pb	Chlorpyrifos	It cause combined toxicity to daphnia magna was preservative	Yongmeng *et al*. (2021)
5	Cd	Diazi on	Seminiferous epithelium, cause loss of spermatogenic element, lacking maturation of germs cells and disorganization	Singh *et al*. (2017)
6	Ni, Mn	Chlorpyrifos	Change in molecular fingerprints and giving rise to a complex transcriptional profile in mixture	Vandenbrouck *et al*. (2009); Boatti *et al*. (2012)
	Cd	Diazinon	progressive damage in epithelial cells and decrease in spermatogenic	Adamkovicova *et al*. (2014)
7	Cd	Chlorpyrifos	Because headaches, seizures, blurred vision, salivation, irritate the stomach, coma, and result in diarrhea and vomiting.	Nandi *et al*. (2022); Mohod and Dhote (2013)
8	Cd	2,4- D, DDT	Tremors or shakiness, vomiting, and seizures. DDT is carcinogen and exposure can affect the liver and reproduction.	Strong *et al*. (2015); Mohod and Dhote (2013)
9	Pb, Cd	Chlorpyrifos	Effect nervous system, blurred vision, ranging from headaches, seizures, coma, and salivation depending on the amount and length of exposure.	Nandi *et al*. (2022); Mohod and Dhote (2013)

*Pb (Lead), Cd (Cadmium), Zn (Zinc), Ni (Nickel), Mn (manganese) and PCB (Polychlorinated biphenyl). A column show combines effect of heavy metals and pesticides.

samples within the limit of WHO. These results are consistent with those of Hange and Awofolu, (2017) who assessed the heavy metal concentrations in river surface water collected from the Jhelum River at Muzaffargarh. The concentrations of Ni, Pb, Cd, and Cr were found higher from WHO limits in the drinking water and metals such as Mn and Zn concentrations were lower acceding to WHO standards for water (Ajiwe *et al.,* 2018). Mohiuddin *et al.* (2016) studied on sediment and water samples from Buriganga river of Bangladesh and found Cd, Pd, Zn, Cu, and As concentration exceeded the toxicity reference values. Mohod and Dhote, (2013) researched that humans sources have been categorized main cause and route in which heavy metals are distributed to different sections of the environment, and transport media are mainly water. In the current study, sources of running water are river Mula, sukhlaji, natural springs and canal water.

These reservoirs are contaminated through natural and anthropogenic actives. Increases in socio-economic actions due to increased population have been connected to the quick heavy metals increase in the environment. Anthropological activities such as agriculture, ore processing, and mining contribute vastly in metal contamination. Domestic activities as laundry detergents also contribute to the heavy metals concentration in water which has contrary effects (Minhas *et al*., 2022). Main water reservoirs of the district Jhal Magsi follow the rout of mountain and agricultural field so there is weather performed the role in the contamination of the water with metals and pesticides (Mustafa *et al*., 2017). According to Akagi, (2024) researched that weathering is a natural procedure that breakdowns rocks, minerals, and soil through atmospheric exposure, living organisms and water. That procedure can release heavy metals, which can cause by numerous natural occurrences such as decay comets, volcanic eruptions, erosion and heavy rainfall. Liu *et al.* (2020) reported higher concentration of Pb and Cd compared to WHO permissible limits in the study conducted on river of Swat water. Briffa *et al*. (2020) studied Hg, Cd, and Pb in surface and ground water of Central East India, his result also shows higher concentration of Pb and Cd in water samples. According to Ajiwe *et al*. (2018), heavy metals have carcinogenic, genotoxic, teratogenic and mutagenic.

Inamori and Fujimoto (2010) conducted research on the safety levels of drinking water by measuring bacteria in 1 mL of test water. Bacterial colonies created were under 100 and that no total coliforms were detected. These results are in contrast of the current study 1 mL of water samples contain the *E. coli* and *P. aeruginosa. E. coli* and *P. aeruginosa* were detected in water by Mustafa *et al.* (2024); Baghal *et al.* (2021) and in tape water (Moghadam *et al*., 2016).

The growth of bacteria is activated by increase in the concentration of phosphorus and nitrogen, commonly found in pesticides. This increase often occurs due to contamination from sources because of industrial sewage, livestock sewage, and domestic sewage which may contain heavy metals. Pathogenic bacteria that cause water-borne diseases can contaminate the human intestine and are runoff into the environment through manure. These bacteria then undergo action and are finally released into water reservoirs, lakes, and rivers (Some *et al.*, 2021).

Cleaning of water involves by using biological agents microbes to eliminate or reduce the effects of environmental pollutants. Microbes are additional utilized mainly because of their fast growth and facility to be simply manipulated, thus their function as agents of bioremediation (Brix, 2020). The current study helps for the coming redressers to study on the role and mechanisms of bioremediation.

The combined pollution of pesticides and heavy metals poses a serious risk to human living and the soil ecological environment. It has been reported that pesticides and heavy metals enter animal and human bodies via ingestion through food materials, dermal contact and inhalation, smoke formation, fumes of chemicals, dust particles, numerous activities, mining, and battery manufacturing (Asgary *et al.*, 2017). The combined pollution of Cu and chlorpyrifos had synergistic and antagonistic effects on different tests (Yongmeng *et al*., 2021). Cd exposure increases the susceptibility to microbial pesticides in a synergistic manner, and the use of microbial pesticides is an effective strategy for pest control in heavy metal-polluted areas (Zheng *et al*., 2023). Microorganisms can convert hazardous Hg^{2+} and Cr^{6+} ions into less toxic (Hg^{0+}) and Cr^{3+} ions (Malik *et al*., 2023). Ni, Cd and chlorpyrifos have synergistic effects on the environment and can cause the conversion of Cd^{2+} and Ni^{2+}. Lam *et al.* (2023) examined the mutual effect of 2,4-dichlorophenol laterally with Cu and Zn.

***Conclusion*:** In the current study pesticides, heavy metals and microbial assessment of water samples were done and were compared with recommend save limits of WHO. From results, it is concluded that DDT (2,4 D), Cyanazine, Chlorpyrifos and Aldrin & Dieldrin pesticides and Pb, Cd were measured higher than WHO standard limits. The predicted synergism resulted from co-existence of the pesticides and the heavy metal represents an enhanced risk to the human further more bacterial assessment help in understanding the role by enhancing the cleaning action and bioremediation process of polluted water.

***Author's contributions*:**

Conceptualization: S.M. and A.-U.-R.K.; **Data curation**: Y.M., S.M. and N.K.; **Investigation**: S.M., A.-U.-R.K., Y.M., N. K., A.S., M.A and S.; **Methodology**: S.M., Y.M. and S.; **Supervision:** A.-U.-R.K., N.K. and A.S.; **Validation**: S.M. and Y.M.; **Writing original draft:** S.M., Y.M. and A.-U.-R.K.; **Writing - review & editing**: N.K., A.S. and M.A

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