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RISK ASSESSMENT OF HEAVY METALS ACCUMULATION AND PREVALENCE OF PATHOGENIC MICROBES IN ORGANS OF *Clarias gariepinus* EXPOSED TO SILVER NANOPARTICLES AND COW DUNG CONTAMINATION

A.L. ADEJUMO[1], L. AZEEZ[2*], W. B. AGBAJE[2], H. K. AREMU[3], A. A. WAHAB[4], M. A. RUFAI[5], A. E. ADELEKE[6], D. F. BAMISAYE[7], H. K. BUSARI[2], V. OGUNGBE[2], A. HAMMED[2]

[1]Department of Chemical Engineering, Osun State University, Osogbo, Nigeria.
[2]Department of Pure and Applied Chemistry, Osun State University, Osogbo, Nigeria.
[3]Department of Biochemistry, Osun State University, Osogbo, Nigeria
[4]Department of Zoology, Osun State University, Osogbo, Nigeria.
[5]Department of Microbiology, Osun State University, Osogbo, Nigeria.
[6]Department of Basic Sciences, Adeleke University, Ede
[7]Department of Biomedical Sciences, Federal Polytechnic, Ede
[*]**Corresponding Author**:luqman.azeez@uniosun.edu.ng, (orcid.org/0000-0002-6415-3490)

ABSTRACT

Using nanomaterials in food packaging and applying cow dung as manure can adversely impact aquatic organisms, with further consequences for human health. Thus, this study investigates metal bioaccumulation, energy requirements and health risks from consumption of *Clarias gariepinus* exposed to silver nanoparticles (AgNPs)-cow dung contaminated water. *C. gariepinus* samples were exposed to clean water (control) and contaminated water with AgNPs (0.75 mg/mL)*, cow dung (0.75 mg/mL) and AgNPs-cow dung (0.75 mg/mL) for 10 days. Cow dung had a comparable load to the control while AgNPs dramatically reduced microbial load in water.* The metal concentration of *C. gariepinus* gills, muscle, kidney, and liver is as follows: Na > K > Mg > Ca > Fe > Al > Zn > Mn > Cr > Cu > Ag > Ni > Pb > Cd > Co. *Metal bioaccumulation was highest in the muscle and lowest in the liver. The estimated daily intake of heavy metals from C. gariepinus consumption was < 0.51, confirming safe consumption.* Non-carcinogenic risks *from consumption of C. gariepinus* exposed to different treatments assessed with hazard index (HI) and target hazard quotients (THQ) were < 1 indicating no non-cancer adverse effects on health. However, calculated life cancer risks (LCR) were higher than the 10^{-4} suggesting possible carcinogenic toxicity from *C. gariepinus* consumption. Creatine kinase activity increased in all study groups, but only the *C. gariepinus* exposed to cow dung showed statistically significant alterations in energy demand. AgNPs considerably decreased the prevalence of gastrointestinal parasites and the bacterial population in *C. gariepinus* organs. These results conclude that the combination of AgNPs and cow manure posed a significant environmental hazard to the survival of *C. gariepinus*.

Keywords: *Clarias gariepinus*, creatinine kinase, heavy metals, microbial population, nanopollution, risk assessment.

INTRODUCTION

Due to their unparalleled functionalities, nanoparticles (NPs) have been used as growth stimulants, fungicides, bactericides, and adsorbents for toxicants in agriculture as part of the drive for innovation and food security (Naasz *et al.,* 2018; Mekkawy *et al.,* 2019; Azeez *et al.,* 2022a,b,c). Additionally, traditional farmers use cow dung as a cheap supply of manure to strengthen and increase the growth of plants (Jena *et al.,* 2020). However, toxicity to aquatic organisms during and after the application of NPs is a valid concern (Turan *et al.,* 2019).
Nanopollution is challenging to control due to the varied sizes, shapes, reactivity, and changes that NPs go through in soil and aqua matrixes. When NPs come into contact with water, they quickly react with the contents. After adopting new properties, these interactions could either accelerate the release of harmful ions and aggregate or reduce their toxicity by increasing their bioavailability (Azeez *et al.,* 2019a,b). Nanoparticles are equally known to bioaccumulate and accelerate metal accumulation in the tissues of fish thereby causing undesirable effects (Tuncsoy, 2022). Silver nanoparticles (AgNPs) are known for their antibacterial, antifungal, and antioxidant characteristics, but they can also be hazardous in their natural state or after being transformed chemically, physically, or biologically (Lateef *et al.,* 2016a,b, 2020; Pikula *et al.,* 2020).

Cow dung, on the other hand, contributes significantly to poor water quality due to the population of microflora (Behera and Ray, 2021).

In addition to their potential toxicity, AgNPs and cow dung may facilitate heavy metal mobilization and bioaccumulation by altering the properties of water bodies (Jena *et al.,* 2020; Shah *et al.,* 2020).

Fish is the cheapest source of animal protein and accounts for 40 % of Nigerian protein consumption. Nigeria is currently the major producer of *Clarias gariepinus*, employing over one million people directly (Azeez *et al.,* 2022b). The fish are mostly reared by partitioning a part of the river usually polluted by contaminants from other agricultural practices. Consequently, they are the most vulnerable aquatic organisms to toxins that limit growth, harm health, and cause pollutants to bioaccumulate in important organs (Amoakwah *et al.,* 2020; Tabrez *et al.,* 2021). As a result, the exposed fish will need more energy to survive (Khan *et al.,* 2016).
Thus, this study investigated the ecological and health risks from bioaccumulation of heavy metals from AgNPs and cow dung contamination in vital organs of *C. gariepinus*. It also studied the energy demands in the exposed *C. gariepinus* and the prevalence of gastrointestinal parasites and microbial populations.

MATERIALS AND METHODS

Green synthesis of AgNPs and preparation of Cow-D

Green synthesis of AgNPs was mediated by adding 1mL of *Prunus dulcis* (almond) leaf extract to 40mL of $AgNO_3$ solution. The resulting AgNPs were monitored for colour change and characterized as reported by Azeez *et al.,* (2022a).

Fresh cow-dung was carefully collected at an abattoir in Oke-Baale Osogbo, Nigeria. The sample was dried for 3 days, pound, sieved and was used for preparation of 0.75 mg/mL cow dung solution.

C. gariepinus acclimation and experimental design

Fresh samples of catfish (*Clarias gariepinus*) were cautiously collected from the fishpond at the Zoological Garden, Osun State University and acclimatized for 3 days in freshwater. The catfishes (1022.74 ± 16.2 g) were randomly grouped into 4 ponds containing four fish in 10 cm × 14 cm ponds holding 100 L water. Group 1 (untreated) served as the control; group 2 was exposed to 450 mL solution of 0.75 mg/mL AgNPs; group 3 received 450 ml solution of 0.75 mg/mL cow dung (Cow-D) while group 4 was exposed to 450 mL solution of a mixture of 0.75 mg/mL each of AgNPs and Cow-D. The fish were fed with 4 % crude protein processed feed (COPPENS). Experimental exposure in each group was done by changing treated (dosed) water after 3 days for 10 days. At the end of the experimental period, all fish were sacrificed 24 h after the last treatment and organs (liver, kidney, muscle and gills) were excised.

Microbiological Analysis of Samples

The sterile swab sticks were used to swab the surface of the intestine, gills, liver and kidney separately and they were inoculated on different nutrient agar plates. The inoculated plates were then incubated at 37 °C for 24 hours. Discrete colonies were sub-cultured into fresh nutrient agar plates aseptically to obtain pure cultures of the isolates. All pure bacterial isolates were Gram stained to determine their gram reaction, followed by other biochemical tests which include Catalase, Oxidase, Mannitol Salt Agar and Deoxyribonuclease (DNase) test and Triple sugar iron test. The morphological characteristics that were examined include, colour, edge, growth, turbidity, elevation, shape and arrangement of microorganisms.

Elemental profile of C. gariepinus, water and cow dung

The elemental compositions of Cow-D, water (control), and treated water (combined after each treatment) were analyzed according to *Azeez et al., (2020)*. Metal contents were determined using an inductively coupled plasma-optical emission spectrometer (ICP-OES) after digesting 0.5 g of each organ with a mixture of HNO_3 and HCl (7:3), following the method described by *Azeez et al., (2020)*.

Health risk assessment

Bioaccumulation factor of metals in C. gariepinus organs

The bioaccumulation of silver and heavy metals in *C. gariepinus* exposed to different treatments was calculated using equation 5.

$$Bioaccumulation\ factor\ (BAF) = \frac{silver/heavy\ metal\ contents\ in\ C.gariepinus}{silver/heavy\ metal\ content\ in\ corresponding\ water} \qquad 5$$

Estimated dietary intake of *C. gariepinus*

The potential risks to the health of humans from consumption of fish (*C. gariepinus*) exposed to nanosilver and cow dung pollution in terms of heavy metals were evaluated using the estimated daily intake (EDI; equation 6) and carcinogenic risks (equations 7 and 8).

$$EDI\ (mgkg^{-1}BWday^{-1}) = \frac{metal\ concentration\ (mgkg^{-1})\ x\ daily\ average\ consumption\ of\ C.gariepinus\ (kg)}{Average\ Body\ weight\ (kg)} \quad 6$$

The daily average consumption of *C. gariepinus* as of 2017 was reported to be 0.062 kg person^{-1} day^{-1} (Liverpool-Tasie *et al.,* 2021). The average body weight of Nigerians was assumed to be 60 kg (Azeez *et al.,* 2020).

Non-carcinogenic and carcinogenic risks

Non-carcinogenic risks in humans from consumption of *C. gariepinus* exposed to polluted water were evaluated using the target hazard quotient (THQ) (equation 7).

$$THQ\ (mgkg^{-1}kg^{-1}BWday^{-1}) = \frac{EDI}{R_fD} \quad 7$$

Where R_fD (oral reference dose) represents the daily allowable dose humans can be exposed to over a period with/without harmful effects. The R_fD for metals of concern are 7, 0.04, 0.0035, 0.003, 0.001, 0.3, 0.02, 0.7 and 0.14 $mgkg^{-1}$ day^{-1} for Al, Cu, Pb, Cr, Cd, Zn, Ni, Fe and Mg respectively (Liu *et al.,* 2019; Rinklebe *et al.,* 2019; Chijioke *et al.,* 2020).

$$HI = \sum THQ \quad 7a$$

Where hazard index (HI) is the sum of target hazard quotients of considered metals. Exposure to toxic metals from consumption of *C. gariepinus* is considered safe with minimal effects if HI and THQ < 1 but when THQ and HI > 1, the probable occurrence of adverse effects from consumption of *C. gariepinus* is high.

Carcinogenic risks from the consumption of *C. gariepinus* exposed to polluted water were calculated using equations 8 and 8a (Chijioke *et al.,* 2020).

Where LCR is the lifetime cancer risks of exposure to toxic metals, CSF is the cancer slope factor for toxic metals and LT is the average lifetime of a Nigerian (48.9 years) (Chijioke *et al.,* 2020). The CSF values of Cr, Cd, Pb and Ni are 0.5, 0.38, 0.01 and 1.7 $mgkg^{-1}$ day^{-1} respectively (Liu *et al.,* 2019; Chijioke *et al.,* 2020). The carcinogenic risk ranges between 10^{-6} and 10^{-4}. The risk is considered negligibly low if CR is lower than 10^{-6}, tolerable between 10^{-6} and 10^{-4} but it is potentially carcinogenic if CR is higher than 10^{-4} (Rinklebe *et al.,* 2019)

Preparation of tissue homogenate

At the end of the experimental periods, the twenty fish samples were sacrificed and tissues were immediately excised, rinsed in 1.15 % KCl and weighed. Homogenization of the tissues was carried out in ice -cold 0.05 M phosphate buffer (pH 7.4) to prepare a 10 % (w/v) homogenate and, subsequently centrifuged at 10,000 rpm for 15 min to obtain supernatants and stored at -4 ° C for metal contents and creatinine kinase activity in muscle.

Creatinine Kinase activity

Creatinine Kinase (CK) activity was assayed in skeletal muscle homogenates based on the colorimetric method described by Hughes (1962). The activity of CK was measured as units of nmol min^{-1} mg^{-1} protein.

Determination of gastrointestinal parasites and bacterial isolates in *C. gariepinus*

Identification of gastrointestinal parasites was done by a complete internal autopsy as described by Charles-River's Research Animal Diagnosis Services. Slides were prepared by smearing crushed and scrapped tissues. A scraping of the entire organ was made in the case of the gills, gall and urinary bladders. Identification of parasites and measurement of parasites infestation were carried out using dissecting microscope.

For bacterial isolation and identification, the swab samples of the gills, liver and kidney were taken. Swab sticks from each organ containing bacterial colonies were inoculated on different nutrient agar and incubated at 37 ^{o}C for 24 h. The discrete colonies were further sub-cultured to obtain pure cultures of isolates. All pure bacterial isolates were subjected to morphological and biochemical characterizations. The biochemical identifications carried out include coagulase, catalase, oxidase, DNase and triple sugar iron tests. The fungal isolates were identified through morphological appearance and microscopic techniques.

Statistical analysis

All results were expressed as mean ± standard error of mean (SEM). Data were tested for normality (Shapiro-Wilk). Differences between treatments and control were carried out using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range post-hoc tests or Kruskal-Walli's test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS (version 25) software package.

RESULTS

Water quality assessment

The effects of AgNPs and cow dung contamination on water quality characteristics show microbial population was significantly reduced in groups treated with AgNPs and AgNPs-cow dung, but the microbial burden was higher in control and cow dung (Table 1).

Table 1: Microbial population in water contaminated with AgNPs, cow dung and AgNPs-cow dung

	Control	AgNPs	Cow dung	AgNPs-Cow dung
Microbial population				
Bacillus spp.	1	1	3	0
Staphylococcus spp.	2	0	0	1
Escherichia coli	0	0	0	1
Klebsiella spp.	0	0	1	0
Salmonella spp.	1	0	0	0
Aspergillus spp.	2	1	2	1
Fusarium spp.	1	0	1	1
Total microbes	7	2	7	4

Elemental contents of contaminated water and ecological risk assessment

The metal contents in cow dung ranges from low (Cr, Cd, Pb, Ni) to moderately high (Fe and Cu) to high (K, Na, Ca, Mg) (Table 2). The AgNPs solution mostly included Ag and certain critical metals in trace amounts, with no heavy metals present (Table 2). Control and AgNPs-contaminated water contained significant levels of important elements (Ca, K, Na, Mg) and moderate levels of Fe, Ag, Cd, Pb, Mn, and Cr. The Cd content in AgNPs-cow dung, as well as Fe and Pb contents in both cow dung and AgNPs-cow dung, were above the permitted limits of 0.005, 0.3, and 0.015 mgL^{-1}, respectively (USEPA, 2018).

Elemental profile of C. gariepinus and health risk assessment

Metal concentrations

The trend of metal contents in gills, muscle, kidney and liver of *C. gariepinus* follows Na > K > Mg > Ca > Fe > Al > Zn > Mn > Cr > Cu > Ag > Ni > Pb > Cd > Co (Table 2). The degree of bioaccumulation of heavy metals and silver in the gills of *C. gariepinus* subjected to only water is as follows: Cu > Pb > Ag > Mn > Ni > Zn > Fe > Cr, while Co and Cd were not observed (Figure 3a). A similar pattern was observed for these metals in muscle. The degrees of bioaccumulation obtained in the gills from AgNPs, cow dung and AgNPs-cow dung follow the trend Fe > Ni > Cd > Cu > Pb > Ag > Cr > Zn > Mn > Co.

Comparable trends were found for AgNPs, cow dung and AgNPs-cow dung bioaccumulation in the muscle with the exception of Pb that had highest degree in AgNPs, Cu in cow dung and Cr in AgNPs-cow dung.

The trend for bioaccumulation of metals in kidney and liver ranged as follows AgNPs > control > AgNPs-cow dung > cow dung following Ag > Fe > Mn > Pb > Ni > Cu > Zn > Cr > Cd (Figure 3b). Metals like Cd and Pb were not found in the kidney and liver of control *C. gariepinus*.

Table 2: Metal concentrations in water, cow dung, AgNPs and organs of *C. gariepinus* exposed to AgNPs, cow dung and AgNPs-cow dung pollution

		Ag	Cd	Co	Cr	Fe	Mn	Ni	Pb	Zn	Cu	Al	Ca	K	Mg	Na
Contaminants (mgkg^{-1})	AgNPs	0.747 ± 0.002^{a}	nd	Nd	nd	nd	Nd	nd	nd	nd	nd	nd	0.002 ±0.000	0.018± 0.000^{a}	0.013 ± 0.000^{a}	0.002± 0.000^{a}
	Cow dung	0.103 ± 0.007^{a}	0.006 ± 0.000^{b}	0.006 ± 0.001^{b}	0.120 ± 0.005^{b}	46.042 ± 1.918^{b}	6.517 ± 0.002^{b}	0.067 ± 0.002^{b}	0.186 ± 0.0131^{a}	1.971 ± 0.075^{b}	19.188 ± 1.623^{b}	4.956 ± 0.03^{b}	4.313 ± 0.03^{b}	42.882 ± 3.816^{b}	51.097 ± 4.322^{a}	20.394± 1.565^{a}
Polluted water (mgL^{-1})	Control	0.017 ± 0.004^{a}	0.003 ± 0.000^{a}	0.001 ± 0.000^{a}	0.062 ± 0.002^{a}	1.376 ± 0.160^{a}	0.244 ± 0.005^{a}	0.019 ± 0.003^{a}	0.003 ± 0.000^{a}	0.603 ± 0.007^{a}	0.016 ± 0.003^{a}	0.493 ± 0.110^{a}	9.031± 0.699^{a}	8.308± 0.227^{a}	5.657 ± 1.052^{a}	17.739± 1.469^{a}
	AgNPs	0.192 ± 0.008^{b}	0.002 ± 0.000^{a}	Nd	0.081 ± 0.003^{b}	2.556 ± 0.050^{b}	1.229 ± 0.003^{b}	0.024 ± 0.000^{a}	0.018 ± 0.004^{b}	1.242 ± 0.014^{b}	0.042 ± 0.002^{b}	0.534 ± 0.082^{b}	9.021 ± 0.033^{a}	21.144 ± 1.088^{b}	6.789 ± 1.113^{b}	20.547 ± 4.561^{b}
	Cow dung	0.108 ± 0.003^{c}	0.006 ± 0.000^{a}	0.008 ± 0.000^{a}	0.219 ± 0.006^{c}	16.229 ± 1.047^{c}	3.837 ± 0.049^{c}	0.171 ± 0.014^{b}	0.024 ± 0.002^{c}	0.918 ± 0.024^{c}	0.151 ± 0.011^{c}	0.378 ± 0.052^{c}	7.642 ± 0.017^{b}	17.457 ± 1.089^{c}	6.303 ± 0.151^{c}	17.315± 4.981^{a}
	AgNPs-Cow dung	0.069 ± 0.003^{d}	0.005 ± 0.000^{a}	Nd	0.174 ± 0.021^{d}	3.948 ± 0.049^{d}	2.796 ± 0.311^{d}	0.063 ± 0.007^{d}	0.029± 0.003^{c}	1.185 ± 0.071^{d}	0.087 ± 0.007^{d}	0.687 ± 0.017^{d}	17.313 ±1.411^{c}	12.582 ± 2.535^{d}	17.702 ± 2.944^{d}	14.510± 1.748^{c}
C. gariepinus gills (mgkg^{-1})	Control	0.011 ± 0.002^{a}	nd	Nd	0.014 ± 0.001^{a}	0.354 ± 0.016^{a}	0.152 ± 0.036^{a}	0.008 ± 0.001^{a}	0.002± 0.000^{a}	0.223 ± 0.074^{a}	0.016 ± 0.002^{a}	0.392 ± 0.008^{a}	4.810± 0.021^{a}	7.002± 0.042^{a}	5.166 ± 0.059^{a}	13.331± 0.497^{a}
	AgNPs	0.045 ± 0.002^{b}	0.001 ± 0.000^{a}	Nd	0.030 ± 0.001^{b}	0.571 ± 0.003^{b}	0.224 ± 0.036^{b}	0.021 ± 0.002^{b}	0.005± 0.000^{a}	0.150 ± 0.004^{b}	0.022 ± 0.004^{b}	0.108 ± 0.001^{b}	1.608± 0.013^{b}	6.122± 0.076^{b}	2.111 ± 0.006^{b}	12.410 ± 1.996^{b}
	Cow dung	0.017 ± 0.003^{c}	0.002 ± 0.000^{a}	Nd	0.014 ± 0.006^{a}	2.545 ± 0.003^{c}	0.326 ± 0.094^{c}	0.022 ± 0.001^{b}	0.004 ± 0.001^{a}	0.143 ± 0.022^{b}	0.020 ± 0.003^{b}	0.179 ± 0.003^{c}	3.074± 0.148^{c}	3.622± 0.055^{c}	5.412 ± 0.052^{c}	10.616± 1.685^{c}
	AgNPs-Cow dung	0.026 ± 0.003^{d}	0.002 ± 0.000^{a}	0.001 ± 0.000	0.070 ± 0.004^{c}	3.633 ± 0.033^{d}	0.444 ± 0.008^{d}	0.022 ± 0.000^{b}	0.005± 0.001^{a}	0.111 ± 0.009^{c}	0.026 ± 0.004^{b}	0.138 ± 0.045^{d}	1.377 ± 0.056^{d}	5.092± 0.596^{d}	1.966 ± 0.074^{d}	12.833 ± 1.266^{d}
C. gariepinus kidney (mgkg^{-1})	Control	0.009 ± 0.003^{a}	nd	Nd	0.028 ± 0.003^{a}	1.118 ± 0.044^{a}	0.160 ± 0.005^{a}	0.007 ± 0.000^{a}	0.001 ± 0.000^{a}	0.214 ± 0.008^{a}	0.014 ± 0.004^{a}	0.386 ± 0.031^{a}	2.796± 0.025^{a}	5.146± 0.202^{a}	4.766 ± 0.098^{a}	10.799± 1.993^{a}
	AgNPs	0.164 ± 0.002^{a}	0.002 ± 0.000^{b}	Nd	0.005 ± 0.000^{b}	1.201 ± 0.005^{b}	0.227 ± 0.003^{b}	0.013 ± 0.000^{b}	0.015 ± 0.000^{a}	0.189 ± 0.003^{b}	0.041 ± 0.001^{b}	0.116 ± 0.003^{b}	1.907 ± 0.054^{b}	7.319± 0.809^{b}	2.646 ± 0.005^{a}	13.810± 3.654^{a}
	Cow dung	0.019 ± 0.001^{a}	0.001 ± 0.000^{b}	Nd	0.023 ± 0.000^{b}	3.036 ± 0.033^{b}	0.307 ± 0.004^{b}	0.013 ± 0.001^{b}	0.019 ± 0.000^{a}	0.172 ± 0.004^{b}	0.021 ± 0.001^{b}	0.447 ± 0.056^{b}	4.842 ± 0.311^{b}	6.184± 0.036^{b}	5.226 ± 0.128^{a}	11.851± 0.993^{a}
	AgNPs-Cow dung	0.024 ± 0.000^{a}	0.002± 0.000^{b}	Nd	0.059 ± 0.007^{b}	3.811 ± 0.401^{b}	0.387± 0.041^{b}	0.019± 0.006^{b}	0.015 ± 0.000^{a}	0.191 ± 0.001^{b}	0.085 ± 0.006^{b}	0.124 ± 0.004^{b}	1.922 ± 0.044^{b}	6.711± 1.111^{b}	2.552 ± 0.893^{a}	13.228± 1.758^{a}
C. gariepinus Liver (mgkg^{-1})	Control	0.005 ± 0.003^{b}	nd	Nd	0.034 ± 0.002^{c}	1.317 ± 0.024^{c}	0.110 ± 0.004^{a}	0.007 ± 0.000^{c}	nd	0.198 ± 0.004^{c}	0.011 ± 0.003^{c}	0.519 ± 0.009^{c}	2.096± 0.094^{c}	8.840± 1.043^{b}	4.236 ± 0.494^{b}	10.423 ± 1.046^{b}
	AgNPs	0.025 ± 0.004^{b}	0.001 ± 0.000^{c}	Nd	0.054 ± 0.002^{d}	1.709 ± 0.066^{d}	0.803 ± 0.039^{c}	0.019 ± 0.003^{c}	0.009 ± 0.001^{c}	0.167 ± 0.009^{d}	0.027 ± 0.007^{c}	0.138 ± 0.009^{d}	1.502 ± 0.007^{b}	7.886± 1.528^{b}	2.369 ± 0.046^{a}	12.276± 2.657^{c}
	Cow dung	0.014 ± 0.002^{c}	0.001 ± 0.000^{c}	Nd	0.015 ± 0.000^{e}	1.565 ± 0.093^{e}	0.358 ± 0.066^{d}	0.008 ± 0.000^{b}	0.015 ± 0.000^{d}	0.162 ± 0.008^{e}	0.022 ± 0.002^{d}	0.024 ± 0.000^{e}	2.034± 0.015^{d}	7.806 ± 1.115^{b}	5.382 ± 0.158^{b}	13.210 ± 1.114^{b}
	AgNPs-Cow dung	0.019 ± 0.002^{a}	0.002 ± 0.000^{a}	Nd	0.091 ± 0.003^{a}	2.950 ± 0.048^{a}	0.224 ± 0.006^{a}	0.012 ± 0.000^{a}	0.017± 0.000^{a}	0.189 ± 0.023^{a}	0.020 ± 0.001^{a}	0.374 ± 0.100^{a}	2.334 ± 0.251^{a}	5.709± 1.854^{a}	2.379 ± 0.241^{a}	11.090± 1.508^{a}
C. gariepinus Muscle (mgkg^{-1})	Control	0.008 ± 0.001^{b}	0.003 ± 0.003^{b}	Nd	0.018 ± 0.002^{a}	0.427± 0.052^{b}	0.155 ± 0.021^{b}	0.010 ± 0.001^{b}	0.003± 0.000^{b}	0.356 ± 0.004^{a}	0.014 ± 0.000^{b}	0.414 ± 0.003^{b}	3.848 ± 0.054^{b}	5.255 ± 0.129^{b}	5.597 ± 0.999^{b}	12.715 ± 2.516^{b}
	AgNPs	0.047 ± 0.004^{c}	0.001 ± 0.000^{bc}	Nd	0.007 ± 0.000^{b}	0.565 ± 0.007^{c}	0.548 ± 0.004^{c}	0.009 ± 0.000^{c}	0.015± 0.000^{c}	0.148 ± 0.002^{a}	0.033 ± 0.003^{c}	0.122 ± 0.008^{b}	1.708 ± 0.051^{c}	8.574 ± 0.046^{a}	3.000 ± 0.009^{c}	15.640± 1.119^{c}
	Cow dung	0.017 ± 0.002^{b}	0.002 ± 0.000^{b}	Nd	0.018 ± 0.002^{c}	1.881 ± 0.067^{d}	0.353 ± 0.011^{c}	0.007 ± 0.000^{a}	0.015 ± 0.000^{d}	0.197 ± 0.009^{b}	0.113 ± 0.000^{a}	10.537 ± 2.051^{b}	2.625 ± 0.802^{d}	6.198 ± 0.809^{c}	5.288 ± 0.802^{d}	12.723± 1.655^{c}
	AgNPs-Cow dung	0.062 ± 0.004^{a}	0.001± 0.000^{b}	Nd	0.103 ± 0.008^{b}	1.267 ± 0.057^{b}	0.261± 0.017^{b}	0.011± 0.000^{b}	0.011 ± 0.001^{a}	0.167 ± 0.015^{b}	0.033± 0.007^{b}	0.662 ± 0.018^{b}	1.686 ± 0.033^{b}	4.313± 0.625^{b}	2.114 ± 1.577^{a}	10.608± 1.440^{a}

Data are presented as mean ± standard error of mean (SEM) of twelve replicates. Different letters indicate significant differences at (p < 0.05) down the row

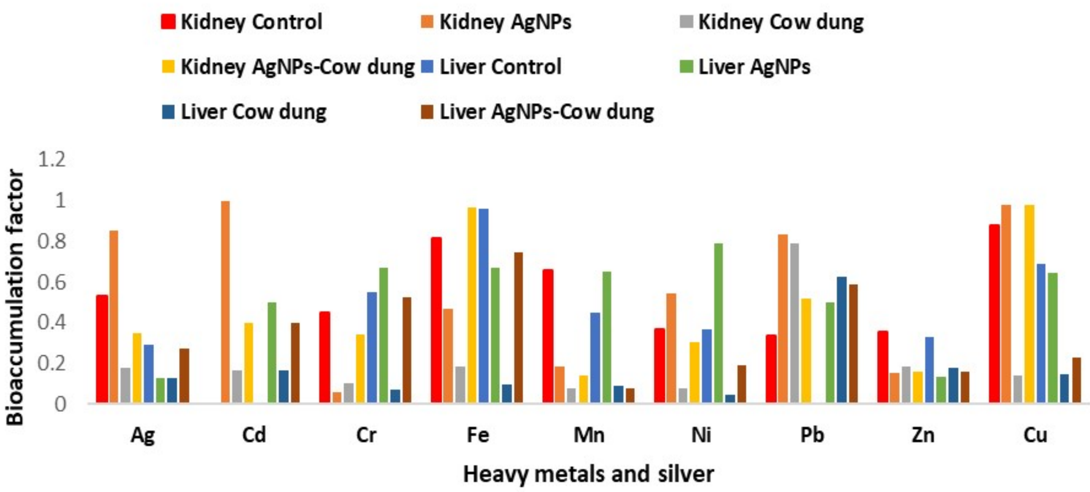

Figure 1a: Bioaccumulation factor of selected heavy metals in gills and muscle of *C. gariepinus* exposed to AgNPs-cow dung polluted water

Dietary intake and health risks

Daily dietary intake of metals associated with health risks via consumption of *C. gariepinus* (Table 3) predominantly in terms of high elemental contents follows AgNPs > AgNPs-cow dung > cow dung > control. Metals with high dietary contents in organs were Mg, Fe, Al, Mn and Zn. All calculated dietary intakes were lower than the allowable limits of different food organizations reported by Chijioke *et al.,* (2020).

Non-carcinogenic risks estimated with hazard index (HI) and target hazard quotient (THQ) were lower than 1 for individual and collective metals in all treatment groups (Table 4). The contribution of metals to THQ follows Mg > Cr > Cd > Pb > Ni > Fe > Cu > Zn > Al in all organs while trend of HI ranges from cow dung > AgNPs-cow dung > control > AgNPs except in liver where AgNPs had higher HI than control.

Carcinogenic risks evaluated with lifetime cancer risk (LCR) were higher than the acceptable tolerable range between 10^{-6} and 10^{-4} implying potential carcinogenicity from the consumption of *C. gariepinus* even in the control (Table 5). The LCR of Pb and Cd were within tolerable limit whereas Cr and Ni were beyond the limit. The CR in gills and liver follow control < cow dung < AgNPs < AgNPs-cow dung, in kidney AgNPs < control < cow dung < AgNPs-cow dung and in m

Table 3: Estimated dietary intake of metals from consumption of organs of *C. gariepinus* exposed to AgNPs, cow dung and AgNPs-cow dung pollution

	Gills				Kidney				Liver				Muscle			
	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung
Ag	1.14E-6	4.65E-5	1.76E-5	2.69E-5	9.30E-6	1.69E-4	1.96E-5	2.48E-5	5.17E-6	2.58E-5	1.46E-5	1.96E-5	8.27E-6	4.86E-5	1.76E-5	6.41E-5
Al	4.05E-4	1.12E-4	1.85E-4	1.43E-4	3.99E-4	1.20E-4	4.62E-4	1.28E-4	5.36E-4	1.43E-4	2.48E-5	3.86E-4	4.28E-4	1.26E-4	5.55E-4	6.84E-4
Cu	1.65E-5	2.27E-5	2.07E-5	2.69E-5	1.45E-5	4.24E-5	2.17E-5	8.78E-5	1.14E-5	2.79E-4	2.27E-5	2.07E-5	1.45E-5	3.41E-5	1.17E-4	3.41E-5
Pb	2.07E-6	5.17E-6	4.13E-6	5.17E-6	1.03E-6	1.55E-5	1.96E-5	1.55E-5	-	9.30E-6	1.55E-5	1.76E-5	3.10E-6	1.55E-5	1.55E-5	1.14E-5
Cr	1.45E-5	3.10E-5	1.45E-5	7.23E-5	2.89E-5	5.17E-6	2.38E-5	6.10E-5	3.51E-5	5.58E-5	1.55E-5	9.40E-5	1.86E-5	7.23E-6	1.86E-5	1.06E-4
Zn	2.30E-4	1.55E-4	1.48E-4	1.15E-4	2.21E-4	1.95E-4	1.78E-4	1.97E-4	2.05E-4	1.73E-4	1.67E-4	1.95E-4	3.68E-4	1.53E-4	2.04E-4	1.73E-4
Cd	2.07E-6	2.07E-6	1.03E-6	1.03E-6	-	2.07E-6	1.03E-6	2.07E-6	-	1.03E-6	1.03E-6	2.07E-6	3.10E-6	1.03E-6	2.07E-6	1.03E-6
Ni	8.27E-6	2.17E-5	2.27E-5	2.27E-5	7.23E-6	1.34E-5	1.34E-5	1.96E-5	7.23E-6	1.96E-5	8.27E-6	1.24E-5	1.03E-5	9.30E-6	7.23E-6	1.14E-5
Fe	3.66E-4	5.90E-4	2.63E-3	3.75E-3	1.16E-3	1.24E-3	3.14E-3	3.94E-3	1.36E-3	1.77E-3	1.62E-3	3.05E-3	4.41E-4	5.34E-4	3.65E-4	2.70E-4
Mg	5.32E-3	2.18E-3	5.59E-3	2.03E-3	4.92E-3	2.73E-3	5.40E-3	2.64E-3	4.38E-3	2.45E-3	5.56E-3	2.46E-3	5.78E-3	3.10E-3	5.46E-3	2.18E-3
Mn	1.57E-4	2.32E-4	3.37E-4	4.59E-4	1.65E-4	2.35E-4	3.17E-4	4.00E-4	1.14E-4	8.30E-4	3.70E-4	2.31E-4	1.60E-4	5.66E-4	3.65E-4	2.70E-4
Co	-	-	-	1.03E-6	-	-	-	-	-	-	-	-	-	-	-	-

Table 4: Non-carcinogenic risk assessment from consumption of *C. gariepinus* exposed to AgNPs, cow dung and AgNPs-cow dung pollution

	Gills				Kidney				Liver				Muscle			
	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung
Al	5.79E-5	1.59E-5	2.64E-5	2.04E-5	5.70E-5	1.71E-5	6.60E-5	1.83E-5	7.66E-5	2.04E-5	3.54E-6	5.52E-5	6.11E-5	1.80E-5	7.93E-5	9.77E-5
Cu	4.13E-4	5.68E-4	5.17E-4	6.72E-4	3.62E-4	1.06E-3	5.43E-4	2.20E-3	2.84E-4	6.98E-4	5.68E-4	5.17E-4	3.62E-4	8.53E-4	2.92E-3	8.53E-4
Pb	5.90E-4	1.48E-3	1.18E-3	1.48E-3	2.95E-4	4.43E-3	5.61E-3	4.43E-3	-	2.66E-3	4.43E-3	5.02E-3	8.86E-4	4.43E-2	4.42E-3	3.25E-3
Cr	4.82E-3	1.03E-2	4.82E-3	2.41E-2	9.64E-3	1.72E-3	7.92E-3	2.03E-2	1.17E-2	1.86E-2	5.17E-3	3.13E-2	6.20E-3	2.41E-3	6.20E-3	3.55E-2
Zn	7.68E-4	5.17E-4	4.93E-4	3.82E-4	7.37E-4	6.51E-4	5.92E-4	6.58E-4	6.82E-4	5.75E-4	5.58E-4	6.51E-4	1.23E-3	5.10E-4	6.79E-4	5.75E-4
Cd	2.07E-3	2.07E-3	1.03E-3	1.03E-3	-	2.07E-3	1.03E-3	2.07E-3	-	1.03E-3	1.03E-3	2.07E-3	3.10E-3	1.03E-3	2.07E-3	1.03E-3
Ni	4.13E-4	1.09E-3	1.14E-3	1.14E-3	3.62E-4	6.72E-4	6.72E-4	9.82E-4	3.62E-4	9.82E-4	4.13E-4	6.20E-4	5.17E-4	4.65E-4	3.62E-4	5.68E-4
Fe	5.23E-4	8.43E-4	3.76E-3	5.63E-3	1.65E-3	1.77E-3	4.48E-3	5.63E-3	1.94E-3	2.52E-3	2.31E-3	4.36E-3	6.30E-4	8.34E-4	2.78E-3	1.87E-3
Mg	3.81E-2	1.56E-2	3.99E-2	1.45E-2	3.52E-2	1.95E-2	3.86E-2	1.88E-2	3.13E-2	1.75E-2	3.97E-2	1.76E-2	4.13E-2	2.21E-2	3.90E-2	1.56E-2
HI	**4.78E-2**	**3.25E-2**	**5.29E-2**	**4.87E-2**	**4.83E-2**	**3.19E-2**	**5.95E-2**	**5.51E-2**	**4.63E-2**	**4.46E-2**	**5.42E-2**	**6.22E-2**	**5.43E-2**	**3.27E-2**	**5.58E-2**	**5.93E-2**

Table 5: Carcinogenic risk assessment from consumption of *C. gariepinus* exposed to AgNPs, cow dung and AgNPs-cow dung pollution

	Gills				**Kidney**				**Liver**				**Muscle**			
	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung
Pb	1.01E-6	2.53E-6	2.02E-6	2.53E-6	5.05E-7	7.58E-6	9.60E-6	7.58E-6	-	4.55E-6	7.58E-6	8.59E-6	1.52E-6	7.58E-6	7.58E-6	5.56E-6
Cr	3.54E-4	7.58E-4	3.54E-4	1.77E-3	7.07E-4	1.26E-4	5.81E-4	1.49E-3	8.59E-4	1.36E-3	3.79E-4	2.30E-3	4.55E-4	1.77E-4	4.55E-4	2.60E-3
Cd	3.84E-5	3.84E-5	1.92E-5	1.92E-5	-	3.84E-5	1.92E-5	3.84E-5	-	1.92E-5	1.92E-5	3.84E-5	5.76E-5	1.92E-5	3.84E-5	1.922E-5
Ni	6.87E-4	1.80E-3	1.89E-3	1.89E-3	6.01E-4	1.12E-3	1.12E-3	1.63E-3	6.01E-4	1.63E-3	6.87E-4	1.03E-3	8.59E-4	7.73E-4	6.01E-4	9.45E-4
CR	**1.08E-3**	**2.60E-3**	**2.24E-3**	**3.68E-3**	**1.31E-3**	**1.29E-3**	**1.73E-3**	**3.17E-3**	**1.46E-3**	**3.02E-3**	**1.09E-3**	**3.38E-3**	**1.37E-3**	**9.77E-4**	**1.10E-3**	**3.57E-3**

The muscle CK activity increased in AgNPs, *Cow-Dung and* AgNPs-Cow-Dung groups (Figure 4). However, the increase was only significant in the fish samples exposed to cow dung as compared to the control ($p < 0.05$).

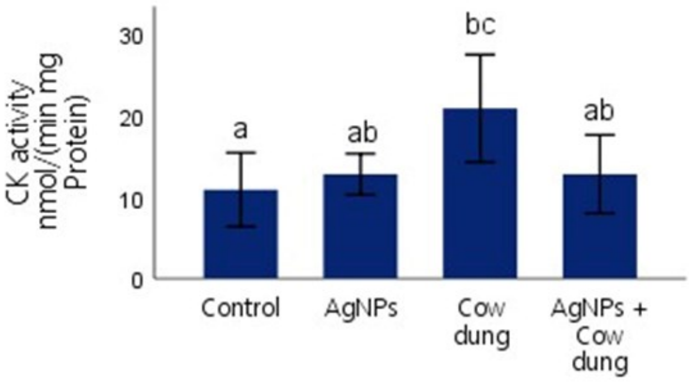

Figure 2: Creatinine kinase (CK) activity in muscle of *C. gariepinus* in the treated groups. Values were expressed as mean ± standard error of mean. Different letters indicate significant difference at ($p < 0.05$)

Prevalence of gastrointestinal parasites and bacterial isolates in *C. gariepinus*

The prevalence and intensity of gastrointestinal parasites in the experimental *C. gariepinus* show a reduction in the index of parasites determined as estimation of parasite count before and after therapy in the stool (Table 6). The reduction in the existing prevalence and intensities of the parasite was observed for 10 days. There was a sharp reduction in the intensity rates after a 10-day exposure to contaminated water. *Trichuris muris* was completely eliminated in *C. gariepinus* exposed to AgNPs. Intensities of *Philometroides africanus* and *Capillaria aerophila* were reduced to 9 and 2 epg from 34 and 41.3 epg respectively (Table 6). All sampled *C. gariepinus* had gastrointestinal parasites at the end of the 10-day treatments but prevalence was observed to reduce according to the species of parasite and the treatment procedure (Table 6a). The highest reduction was recorded in AgNPs followed by AgNPs-cow dung. Control and cow dung treatments had the lowest reduction rates. Overall, there was no significant difference in the overall antiparasitic activities ($p > 0.05$).

In addition, a total of 35 bacterial isolates were identified from the internal organs of the *C. gariepinus*. These isolates range across seven genera namely, *Bacillus* spp., *Staphylococcus* spp., *Escherichia coli, Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., *Streptococcus* spp. (Table 7). Considering organs treated with AgNPs, bacteria colonization was reduced compared to control and cow dung treated groups. *Klebsiella* spp. and *Escherichia coli* were not identified in *C. gariepinus* exposed to AgNPs.

Table 6: Mean Prevalence and Intensities of gastrointestinal parasites in the experimental *C. gariepinus* before and after treatments.

Parasites	Mean Prevalence (%) and intensities (epg) before treatment	Mean Prevalence (%) and intensities (epg) of parasites after treatment			
		Water (control)	AgNPs	AgNPs-Cow dung	Cow dung
Trichuris muris	(100) 25.7	(100) 22.5	(100) 0	(100) 12	(100) 33.6
Philometroides africanus	(100) 34.0	(100) 30.0	(100) 9.0	(100) 14.0	(100)18.0
Capillaria aerophila	(100) 41.3	(100) 26.0	(100) 2.0	(100) 5.0	(100) 41.0

Table 7: Percentage of bacterial isolates in *C. gariepinus*

	Gills				Kidney				Liver				Muscle			
	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung	Control	AgNPs	Cow dung	AgNPs-Cow dung
Staphylococcus spp.	0	0	0	0	0	0	0	0	0	1	3	2	0	0	0	0
Salmonella spp.	0	0	2	0	0	0	0	0	0	0	0	0	3	1	2	0
Bacillus spp.	0	0	0	2	0	0	3	0	0	0	0	0	0	0	0	0
Pseudomonas spp.	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Klebsiella spp.	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Streptococcus spp.	0	1	0	0	0	0	0	0	0	1	0	0	0	2	0	0
Total	5	1	6	2	0	0	3	0	0	2	3	2	3	3	2	0

DISCUSSION

The decrease in the microbial population in AgNPs-contaminated water could be attributed to AgNPs' antibacterial capabilities. Fungi, bacteria, and parasites have all been shown to be inhibited by AgNPs (Lateef *et al.,* 2016a, 2016b; Turan *et al.,* 2019).

The diverse metal concentrations, contamination factors, contamination degree, and PLI in contaminated water, particularly in cow dung and AgNPs-cow dung, indicate that the treated water was exceedingly contaminated with heavy metals. Continuous exposure of *C. gariepinus* to these sorts of contaminated water may cause an imbalance in the performance aquatic organisms, as heavy metal exposure is clinically connected with fish death and functional obstacles (Tuncsoy, 2022). Furthermore, persistent exposure to low levels of these metals may change biochemical and haematological markers in aquatic creatures (Adewumi and Laniyan 2020; Amoakwah *et al.,* 2020; Tabrez *et al.,* 2021). The ecological consequences of various contamination processes, as assessed by risk assessment, demonstrate that cow dung posed the most harmful ecological dangers under both the worst and best conditions, while the control posed no concern. The biggest threats were accounted for by the Fe and Pb contents in all treatment groups. The Pb posed a greater threat to aquatic organisms and the environment since it is known to disrupt the immune system and key organs in fish (Azeez *et al.,* 2020; Chijioke *et al.,* 2020).

The presence of non-essential elements and their possible consumption through *C. gariepinus* ingestion were both below suggested limits. Heavy metals in control water may be due to fish feeds, as it has already been discovered that some metals are utilized as supplements in commercial fish meals (Chijioke *et al.,* 2020). Furthermore, heavy metal contents in *C. gariepinus* organs were lower than those reported by Khan *et al.,* (2020) for *Oreochromis niloticus* and Tabrez *et al.,* (2021) for *Mystus vittatus, Mystus tengara,* and *Mystus tengara*. Although the bioaccumulation of most non-essential metals was <1; Pb, Cd, Cu, and Fe exhibited bioaccumulation of approximately 1 in all organs, indicating the possibility of toxicities being induced by exposure to metals (Islam *et al.,* 2019; Chijioke *et al.,* 2020). Recent studies have shown that elevated Cu, Cd, Cr, Al, and Fe concentrations were toxic to *Pampus chinensis, Oreochromis niloticus, Mugil cephalus, Ctenopharyngodon idella, Tenualosa toil, Catla catla, Liza parsia,* and *Hyporhamphus limbatus* (Abdel-Khalek *et al.,* 2016; Ahmed *et al.,* 2019; Khan *et al.,* 2020; Shah *et al.,* 2020; Naz *et al.,* 2021). Muscle has the highest likelihood of bioaccumulation, indicating that it might have suffered the most negative effects from heavy metals. This could be because it has lower metabolic activity than the liver and kidney. Furthermore, this may be attributed to direct adsorption on muscle tissues (Abdel-Khalek *et al.,* 2016; Ahmed *et al.,* 2020; Tuncsoy, 2022).

For all metals of concern, estimated dietary intake (EDI) was lower than the recommended daily allowance (Islam *et al.,* 2019; Chijioke *et al.,* 2020). Additionally, the possibility of these metals playing roles in causing non-carcinogenic risks was < 1, implying that there is a low risk of health hazards from consuming *C. gariepinus* exposed to various treatments. Continuous consumption of *C. gariepinus* exposed to varied treatments, on the other hand, may offer substantial health risks despite having lower toxicity as determined by the non-carcinogenic risk assessment. The estimate of carcinogenic hazards from *C. gariepinus* consumption exposed to these treatments over a lifetime would constitute a possible hazard if the findings as obtained in this study are > 10^{-4}. This could be owing in part to high cancer slope factor of Ni and the combined impacts of other heavy metals, particularly Cr (Islam *et al.,* 2019; Azeez *et al.,* 2020; Chijioke *et al.,* 2020).

Creatinine kinase (CK) is one of the energy -related factors that perform integrated activities such as energy buffering, metabolic capacity, energy management, and transfer, all of which help to maintain appropriate energy homeostasis (Paula *et al.,* 2009). Muscle CK activity increased in all experimental groups in this study, but only the Cow-D group was significant. This could be due to an increase in energy needs for detoxification mechanisms needed to reduce the impact of toxicants. The results of *in vivo* exposure of *C. gariepinus* to AgNPs alone and AgNPs plus cow dung indicate that the energy reserve was not significantly affected. The energy need is determined by the organism's state (Khan *et al.,* 2016). This work also suggests that the greatly enhanced CK activity found in *C. gariepinus* exposed to cow dung may have disrupted energy homeostasis, resulting in an urgent need to maintain ATP and ionic gradient concentrations to meet the energy need under chemical stress.

At the end of the 10-day treatment, all of the analyzed fish had gastrointestinal parasites, but the prevalence varied according to parasite species and experimental group. *C. gariepinus* subjected to AgNPs demonstrated significant decrease, followed by the AgNPs -cow dung group. The reduced incidence of parasites in AgNPs was connected with their antibacterial and antiparasitic characteristics (Turan *et al.,* 2019; Lateef *et al.,* 2020), but pond water deterioration by cow dung had highest number of parasites which is consistent with the results of Khan *et al.,* (2018), Jena *et al.,* (2020), and Behera and Ray (2019). (2021). The reduction in the population of bacteria isolated in the organs of *C. gariepinus* exposed to AgNPs is consistent with the findings of Geetha et al (2014), who found that AgNPs were effective against *Klebsiella*, *Escherichia coli*, and other bacterial species such as *Pseudomonas fluorescence, Pseudomonas aeruginosa, Shigella flexaneri* and *Proteus mirabilia.* The high percentage of bacteria collected from the gills could be due to frequent contact with the aqueous environment, and it has previously been noted that the gill is known to house a large number of bacterial isolates (Meron *et al.,* 2020).

CONCLUSION

This study described the pollution of water by AgNPs and cow dung, as well as the effects on water quality. The trends of risk quotients and the pollution load index are influenced by AgNPs < AgNPs-cow dung < cow dung. Contamination from these sources significantly affected water quality, according to risk quotients and ecological disturbance. The presence of gastrointestinal parasites and bacterial populations in *C. gariepinus* organs was considerably reduced by AgNPs. Cow manure, in particular, caused a considerable shift in energy requirement in *C. gariepinus.* As a result, using metal nanoparticles in everyday products and cow dung as fertilizer should be done with prudence.

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