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Sensitivity of *Daphnia magna*: Acute Toxicity Evaluation of 22 Metals

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Abstract:
Introduction: All potentially toxic metals in the environment can be discharged in the aquatic ecosystems. *Daphnia magna* is one of the most sensitive species to toxic chemicals in water and is frequently used in toxicological research and environmental monitoring.

Material and Methods: Acute toxicity test for twenty-two metals (Pb, Cd, Ni, Hg, Cu, Fe, Co, As, Cr, Mn, Zn, Al, Pd, Na, K, Mo, Mg, Ca, W, Ir, Ti, Ag) was carried out for *Daphnia magna*. These elements were checked and ranked in terms of decreased immobilization (EC_{50}) after 24Hr and 48Hr and compared with previous studies.

Results: The results were categorized into four groups by the 48Hr EC50s values: highly toxic groups (Ag, Hg, Cu, Cd, Pd) [EC50 < 100 µg.l^{-1}], moderately toxics groups Cr, Fe, Ni, Zn and Pb) [100 µg.l^{-1}<EC50 < 1 000 µg.l^{-1}], low toxic groups (Al, Mn, As, Ti, Co, W and Ir) [1000 µg.l^{-1}<EC50 < 100000 µg.l^{-1}], and minimally toxic groups (Na, Mg, K, Ca, and Mo) [EC50 > 100 000 µg.l^{-1}]. Correlation coefficients (*r*) between EC values and eight physicochemical properties were also examined. The results obtained in this study were weak.

Conclusion: This work adds and confirm data about the toxicities of metals in aquatic ecosystems by using a rapid biomonitoring test.

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1. INTRODUCTION

Metals are essential for human life and physiological functions. However, some are toxic [1-3]. These metals can produce reactive oxygen species (ROS) and induce oxidative stress in cells. ROS attack macromolecules (e.g., protein, lipid, and DNA), leading to disease, ageing, and cell death [4].

With rapid industrialization and a new human lifestyle, metals are increasingly used and dropped in aquatic environments making water pollution as one of the most severe problem in the world [5, 6].

Daphnia magna, a freshwater microcrustacean of the order Cladoceran, plays an essential role in the matter and energy fluxes as a primary consumer in the aquatic ecosystem [7]. Hence, it is one of the most vulnerable organisms to toxic chemicals in water bodies such as metals or herbicides [8]. This species is recommended as a model in several standardized guidelines for chemical risk assessment [9]. Small size, short life cycle, availability, high fertility, and mode of reproduction by parthenogenesis, make of Daphnia magna frequently chosen in aquatic ecotoxicology tests [10].

Many studies conducted metals acute toxicity on Daphnia magna and calculated the correlation between acute toxicity and the physicochemical constants [11-13]. This test was performed under a unified condition (pH, hardness, salinity) for the intercomparison of metal toxicities. Our work aligns with previous ones and want to complete information by testing 22 metals (*Na*, *Mg*, *Al*, *K*, *Ca*, *Ti*, *Mn*, *Cr*, *Fe*, *Co*, *Ni*, *Cu*, *Zn*, *As*, *Mo*, *Pd*, *Ag*, *Cd*, *W*, *Ir*, *Hg*, and *Pb*).

This work should provide helpful information about the toxicity of metal in aquatic ecosystems by (1) comparing results with previous studies, (2) examine the effect of hardness on the metals toxicities, and (3) look for a correlation between physicochemical constants and toxicity of metals.

2. MATERIAL AND METHODS

2.2. *Daphnia magna* Culture

Daphnia magna individuals were collected from a natural water region in Taref, Algeria, then, were subcultured at the Technical Platform in Physico-chemical Analysis, Ouargla, Algeria (PTAPC-Ouargla). Female neonates (age <24Hr), produced by mature females (age ≥ two weeks), were subcultured every week. Neonates *Daphnia magna* was used for experiments after the acclimation of several generations (Figure **1**). Water temperature for breeding was 21±0.5°C, and the light/dark cycle was 16/8Hr. Breeding was conducted in glass flasks using 50 adult specimens per litre. The Daphnia were fed with a spinach solution four days per week.

2.2. Chemicals and Test Solutions

In this study, two types of stock solutions were prepared for testing, each following distinct preparation methods. Some were prepared as 1000 mg/L standard solutions of each element in liquid form: Magnesium

Figure 1: Microscopy images of (**A**) Adult *Daphnia magna*, (**B**) 24Hr-old *Daphnia magna* x 5 magnification.

(Merck), Calcium (Merck), Titanium (Merck), Manganese (Merck), Iron (Merck), Cobalt (Merck), Nickel (Merck), Copper (Merck), Zinc (Merck), Molybdenum (Merck), Palladium (Merck), Cadmium (Merck), Iridium (Bernd Kraft), Mercury (Perkin Elmer), and Lead (Merck). Additionally, specific chemical powders were dissolved directly in the breeding water to create other solutions; these included As_2O_5, *NaCl*, *KCl*, $Al(NO_3)_3$, K_2CrO_4, $AgNO_3$, and $NaWO_4{\cdot}2H_2O$. The solutions were each mixed with the breeding water for 24 hours. After this mixing period, the supernatant was collected and used for further studies.

2.3. Acute Toxicity Testing

The toxicity tests were performed according to OECD (2004) guidelines. Five test concentrations were used for each metal. The dilution factor was two, and the dilution water used was filtered, and aerated (hardness 100 mg.l^{-1} as $CaCO_3$). Experiments were conducted in an incubator at 21±0.5°C, 16Hr light/8Hr dark, and pH 6.5–8.5. Breeding water was used for test water.

Five females neonates (age<24h) with four replicates were introduced into a glass tube test for each concentration. There was no feeding during the test, and the containers were slightly aerated. After 24Hr and 48Hr, the mobile ones were counted after gently shaking the glass containers, which could not move after 10 seconds were regarded as immobile. The toxicity is expressed by the initial concentration that inhibits the mobility of 50% of the daphnids during a 24Hr and 48Hr period of exposure.

24Hr and 48Hr EC_{50s} for each metal (median effective concentration) were calculated using a regression line obtained by plotting the concentration (on a logarithmic scale) against the immobilization percentage on a probit scale, and the results were estimated with probit analysis. The concentration was expressed in µg.l^{-1}.

2.4. Chemical and Statistical Analyses

pH and dissolved oxygen concentrations were determined at the start and end of the tests. No significant changes between pH before and after exposure were recorded. All EC_{50s} values were transformed into respective molar concentrations per litre and converted into negative logarithms (pM). The coefficient of correlation (*r*) was determined between

Table 1: Acute Toxicity of Metals to *D. magna*

Compound	Purity (%)	48 hr		
		EC_{50} (µg.L^{-1})	Molarity	pM (-Log Molarity)
NaCl	99.5	2,904,361	1.26 x10^{-2}	0.90
$MgSO_4$. $5H_2O$	Standard solution	160,000	6.58 x10^{-3}	2.18
$Al(NO_3)_3.9H_2O$	98.0	3,800	1.41 x 10^{-4}	3.85
KCl	99.0	376,142	9.62 x 10^{-3}	2.02
$Ca(NO_3)_2$	Standard solution	120,000	2.99 x 10^{-3}	2.52
$Ti(NO_3)$	Standard solution	1,835	3.83 x 10^{-5}	4.42
KCr_2O_4	99.0	322	6.19 x 10^{-6}	5.21
$Mn(NO_3)$	Standard solution	9,500	1.73 x 10^{-4}	3.76
$Fe(NO_3)$	Standard solution	519	9.29 x 10^{-6}	5.03
$Co(NO_3)$	Standard solution	1,749	2.97 x 10^{-5}	4.53
$Ni(NO_3)$	Standard solution	510	8.69 x 10^{-6}	5.06
$Cu(NO_3)$	Standard solution	63	9.96 x 10^{-7}	6.00
$Zn(NO_3)$	Standard solution	161	2.46 x 10^{-6}	5.61
As_2O_5	99.0	1,411	1.88 x 10^{-5}	4.73
$Mo_7O_{24}(NH_4)_6$	Standard solution	1,400,000	1.46 x 10^{-2}	1.84
$Pd(NO_3)$	Standard solution	2,795	2.63 x 10^{-5}	4.58
$Ag(NO_3)$	99.0	0.22	2.01 x 10^{-09}	8.70
$Cd(NO_3)$	Standard solution	61	5.42 x 10^{-07}	6.27
$NaWO_4.2H_2O$	99.0	46,765	2.54 x 10^{-4}	3.59
$Ir(NO_3)$	Standard solution	1,969	1.02 x 10^{-5}	4.99
$Hg(NO_3)$	Standard solution	0.43	2.13 x 10^{-9}	8.67
$Pb(NO_3)_2$	Standard solution	181	8.72 x 10^{-7}	6.06

negative logarithms (pM) of metals, and physicochemical constants included are electronegativity, atomic weight, melting point, boiling point, thermal conductivity, and ionization potential.

3. RESULTS

3.1. Acute Toxicity Test Validation

According to OECD (2004) [9], the test met the validity criteria: control samples showed no more than 10% effect, and dissolved oxygen concentration at the end of the test remained above 3 mg/L.

3.2. Metals Toxicities

Data of 48Hr EC$_{50}$s were calculated. All EC$_{50}$ values were transformed into respective molar concentrations per litre and converted into negative logarithms (pM) (Table **1**).

The most toxic element was Ag with 48Hr EC$_{50}$: 0,22 µg.l^{-1} and the less harmful was Na with 48Hr EC$_{50}$: 2 904 361 µg.l^{-1}.

The results were categorized into four groups by the 48Hr EC$_{50}$s values: highly toxic metals to *Daphnia magna* (Ag, Hg, Cu, Cd, Pd) (EC$_{50}$ < 100 µg.l^{-1}), moderately toxics metals (Cr, Fe, Ni, Zn and Pb) (100 µg.l^{-1}<EC$_{50}$ < 1 000 µg.l^{-1}), low toxic groups (Al, Mn, As, Ti, Co, W and Ir) (1 000 µg.l^{-1}<EC$_{50}$ < 100 000 µg.l^{-1}), and minimally toxic groups (Na, Mg, K, Ca, and Mo) (EC$_{50}$ > 100 000 µg.l^{-1}).

Sensitivity comparison between EC$_{50}$s in this study and EC50s [11-13], was conducted respectively for 21, 12, 19, and 11 common metals (Table **2**). The

Table 2: Acute Toxicity Values used for the Comparison of Species Sensitivities

Metals	**48 hr EC$_{50}$ (µg/L)**			
	D. magna **(This study, hardness, 100 mg.L^{-1})**	***D. magna*** **(hardness, 72 mg.L^{-1})^{a}**	***D. magna*** **(hardness, 45 mg.L^{-1})^{b}**	***D. magna*** **(hardness, 240 mg.L^{-1})^{c}**
Na	2,904,361	1,600,000	1,640,000	423,130
Mg	160,000	140,000	140,000	288,720
Al	3,800	930	3,900	50,230
K	376,142	340,000	93,000	160,450
Ca	120,000	870,000	52,000	560,000
Ti	1,835	5,700	-	-
Cr	322.18	130		1,840
Mn	9,500	9,300	9,800	10,270
Fe	519	2,300	-	2,920
Co	1,749	710	-	1,670
Ni	510	650	510	5,700
Cu	63	13	9.8	14
Zn	161	720	100	130
As	1410.86	2,400	7,400	74,000
Mo	1,400,000	1,500,000	-	78,080
Pd	27.95	-	-	-
Ag	0.22	0.91	-	14
Cd	61	3.6	65	80,770
W	46,765	30,000	-	65,180
Ir	1,968	3,000	-	-
Hg	0.43	0.65	5	22
Pb	181	290	450	8,690

[a] Data collected from Okamoto *et al.*, 2015 [11].
[b] Data collected from Biesinger and Christensen., 1972 [12].
[c] Data was collected from Kagharot *et al.*, 1989 [13].

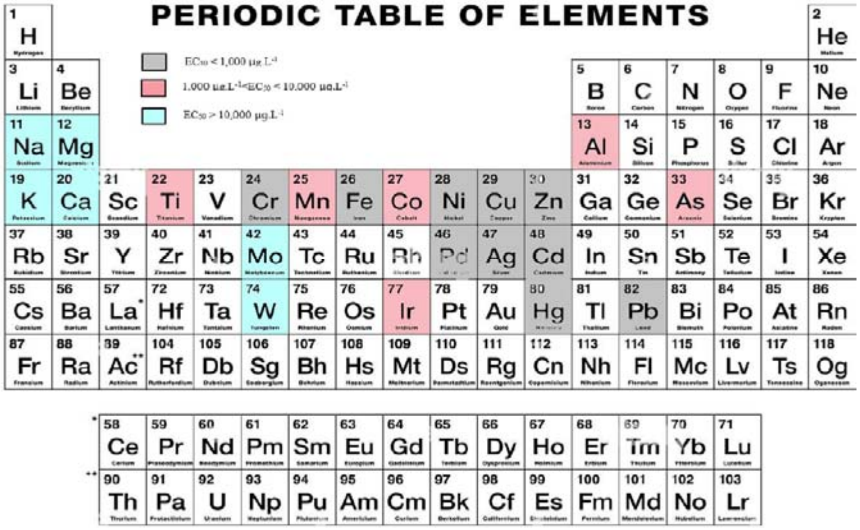

Figure 2: Acute toxicity metals classification to *D. magna*.

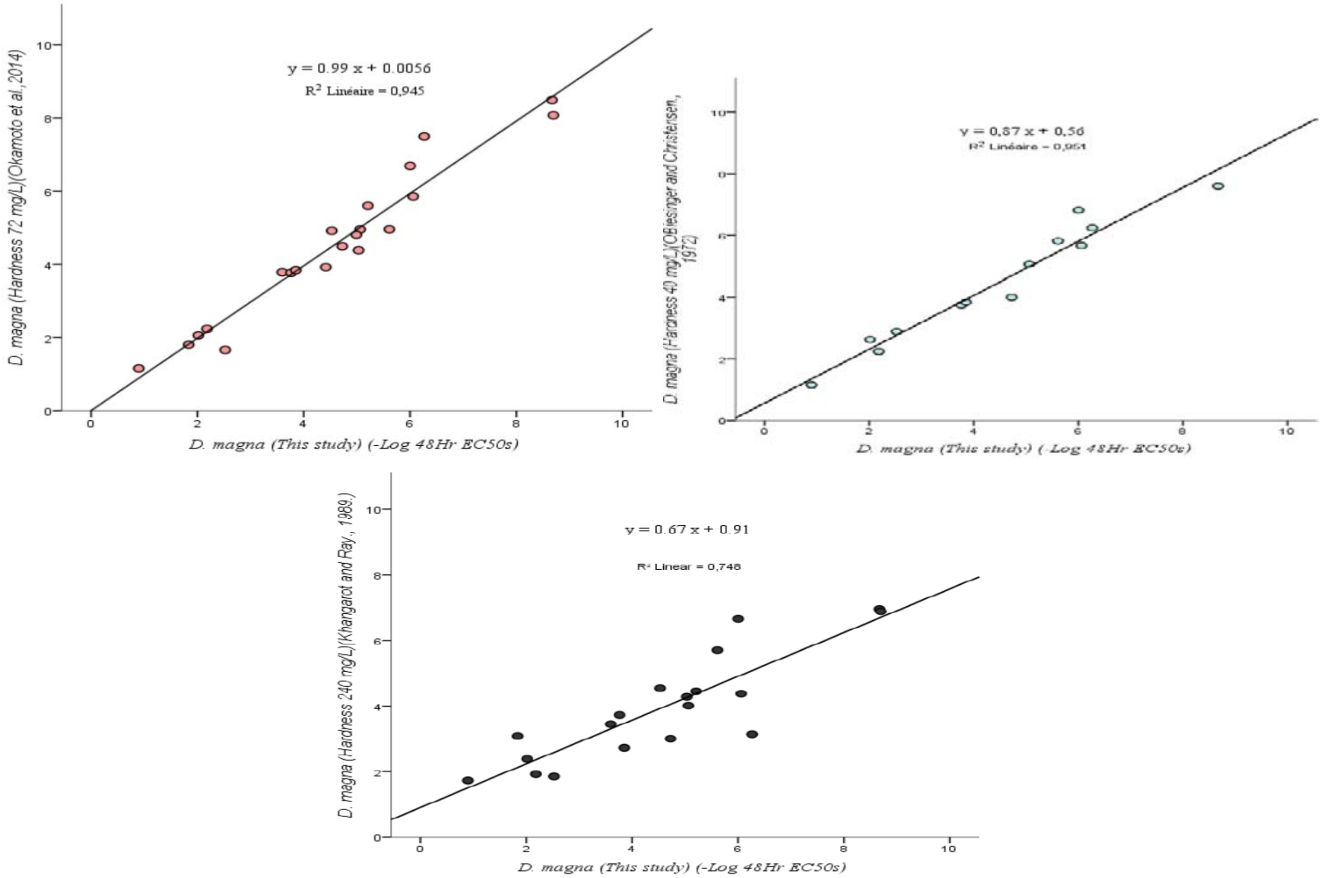

Figure 3: Correlation between the EC_{50} values in our study and those of previous investigations, as described by the least-squares solid line: (A) *D. magna* (hardness: 72 $mg.L^{-1}$); (B) *D. magna* (hardness: 45 $mg.L^{-1}$); (C) *D. magna* (hardness: 240 $mg.L^{-1}$).

determination coefficient was 0.94, 0,95, 0.74 and 0,89 (Figure **2**).

Table **3** ranks the 48Hr EC_{50}s of tested metals in descending order in our study and older studies.

Table **4** shows the correlation between physicochemical proprieties of metals and our results for 48Hr EC50 for 22 elements.

Table 3: Classification of Metal Toxicity to Daphnia

Studies	Decreasing order of metals toxicity
Our study	Ag>Hg>Cd>Cu>Zn>Pb>Ni>Fe>Co>Ti>Al>Mn>Ca>Mg>K>Mo>Na
Okamoto et al, 2015	Hg>Ag>Cd>Cu>Pb>Ni>Co>Zn>Fe>Al>Ti>Mn>Mg>K>Ca>Mo>Na
Biesinger and Christensen, 1972	Hg>Cu>Cd>Zn>Pb>Ni>Al>Mn>Ca>K>Mg>Na
Khangarot and Ray, 1989	Cu>Ag>Zn>Co>Fe>Ni>Pb>Mn>Al>Mo>Cd>K>Mg>Na
Cui et al, 2018	Ag>Hg>Cd>Cu>Zn>Pb>Fe>Ni>Mn

Table 4: Correlation between the Physicochemical Proprieties of Metals and our Results for 22 Elements

Physicochemical propriety	correlation coefficient (r)
Relative atomic mass	0.530
Melting point	-0.140
Boiling point	-0.220
Electronegativity	0.452
Enthalpy	-0.142
enthalpy vaporization	-0.079
Thermical capacity	-0.666
Thermical conductivity	0.132

4. DISCUSSION

4.1. Effect of Hardness on Metals Toxicities

Proprieties of water (pH, hardness, salinity, and temperature) used in *D. magna* acute toxicity test are responsible for differences in values of metals toxicity [14-17].

The determination correlation of our 48Hr EC_{50}s (hardness: $100mg.l^{-1}$) and okamoto and beisinger study (hardness: $45mg.l^{-1}$), (hardness: $72mg.l^{-1}$) was greater than kagharot study (hardness: $240\ mg.l^{-1}$).

These results show that the toxicity of metals was highly increased in the low hardness solution. Yim *et al*. (2006) [14] have the hardness of a solution cause different toxicity, that is, higher toxicity in a soft than a hard test solution. We suppose that the calcium concentration explains this phenomenon. Tan and al. Have demonstrated that Calcium decreases cadmium toxicity by minimizing its absorption [18].

As shown in other studies, it is clear that hardness is a significant factor affecting metal toxicity and should be considered in determining reference EC_{50}s values [19, 20].

In another way, the correlation between EC50s in our study and this of *D. galeata* was high. This suggests that *D. magna* is sensitive enough to be used in aquatic ecotoxicity tests for daphnia studies. Cui *et al.* (2018) [21] indicate that *D. galeata* is a suitable species for marine ecotoxicity tests because of its higher sensitivity to metals [21].

Mechanisms of metals toxicity are not equivalent even in Daphnia. Therefore, further study is needed to investigate the toxic mechanisms of many metals.

4.2. Classification of Metals was Categorized in Groups

Highly toxic groups (Ag, Hg, Cu, Cd, Pd) (48Hr $EC_{50}<100µg.l^{-1}$).

Elevated silver (Ag) concentrations in water are associated with anthropogenic activities such as mining and photographic processing. Daphnids exposed to Ag exhibited ionoregulatory disturbance, which was characterized by decreases in whole-body sodium concentration. This was explained by competitive inhibition of the whole-body sodium uptake [22].

Mercury (Hg) compounds are widely distributed pollutants and can cause toxicity to kidneys, livers, and lungs [23]. They may bring danger to the growth and development of aquatic organisms [24]. The mechanisms are based on its chemical activity and biological features, suggesting that oxidative stress is involved in its toxicity [1]. Through oxidative stress, mercury has shown mechanisms of sulfhydryl reactivity. Once in the cell, Hg^{2+} and MeHg form covalent bonds with cysteine residues of proteins and deplete cellular antioxidants [25].

Palladium pollution is increasing due to automobile traffic and is introduced into aquatic biotopes where they accumulate in sediments of lakes and rivers. The concentration found in our study was similar to Zimmermann *et al*. (2017) [26] study that shows palladium as highly toxic to *D. magna* [26].

Cadmium is highly toxic and is released in the environment due to its direct or indirect use in various industrial applications [27]. It is nephrotoxic metal and may be related to multiple cancers [28]. Cd may endanger the growth and development of aquatic life by its potency to alter ion homeostasis, bind sulfhydryl groups and modulate the properties of many proteins and enzymes and generate oxidative stress [29-31].

Copper (Cu) concentrations have steadily increased in aquatic environments owing to agrochemical usage, industrial waste emissions, and mining activities [32]. Cu toxicity is explained by ROS formation that causes oxidative stress and ionoregulatory disturbance to freshwater osmoregulating molluscs [11, 33, 34].

Moderately toxics groups Cr, Fe, Ni, Zn and Pb) (100 µg.l-1<EC50 < 1 000 µg.l-1), Lead (Pb) is a non-essential, non-biodegradable, and highly toxic metal to many organisms, even at low concentrations [12, 35]. The carapax of the dead daphnids was abraded, and in higher concentrations, partial ruptures were observed mainly in the carapax. Pb inhibits waterborne Ca^{2+} uptake in a concentration-dependent manner by a direct competitive interaction between Pb^{2+} and Ca^{2+} at the epithelial surface [36].

Nickel (Ni) is an essential aquatic contaminant present at elevated concentrations in many areas engaged in mining-related activities [37]. Ni is toxic to D. magna by Mg^{2+} antagonism [38].

Zinc (Zn) toxicity to Daphnia magna is hypothesized by a disturbance of Ca balance [39].

Seven 48Hr EC_{50} metals (Co, Ti, As, W, Ir, Al, and Mn) were between 1000 $µg.l^{-1}$ and 100000 $µg.l^{-1}$.

Iridium is mainly used in metallurgy as a hardening agent in very stable alloys of platinum in its metallic form is generally not toxic due to its non-reactivity but should be monitored.

Arsenic is known to induce acute and chronic adverse effects on the health of humans and other organisms. The exposure of D. magna to arsenic causes oxidative stress. As (III) has a high affinity with sulfhydryl groups and leads to conformation changes in proteins, eventually influencing enzyme activity [40].

Five 48Hr EC_{50} metals (Na, K, Ca, Mo and Mg) were not toxic to D. magna (> 100000 $µg.l^{-1}$) and belonged to groups 1 and 2 in the periodic table. This low toxicity can be explained by the fact that these metals are essential for good cell function.

The acute toxicity results of 15 elements (Na, Ca, Mg, K, Sr, Ba, Mn, As, Al, Zn, Ni, Cu, Co, Hg, and Cd) obtained by Biesinger and Christensen (1972) [12] were correlated with electronegativity, and the correlation coefficient was 0.72. Okamoto *et al*. (2015) [11] did not get a correlation for 50 metals. The 22 elements were not correlated with electronegativity, and the correlation coefficient was 0,45. Therefore, we revealed that the toxicity of metal was not related to its electronegativity.

Moreover, we examined the relationship between other physicochemical constants and toxicity that shown in Table **4**. The correlation coefficient between metal toxicities and first atomic weight was 0.52, indicating that acute toxicity results were not correlated with nuclear weight. Similarly, the correlation coefficient between acute toxicity and atomic weight was 0.47. In our study, the sensitive toxicity results of metals were also not correlated with physicochemical constants included are electronegativity, atomic weight, melting point, boiling point, thermal conductivity, and ionization potential. Therefore, the toxic functions of metals are complex.

5. CONCLUSION

To conclude. Understanding the problems associated with the degradation of water quality requires detailed knowledge of the state of an aquatic system and how it changes with time. The development of new methods that can be used to identify the presence of toxic substances that affect water quality is significant to guarantee a continuous supply of high-quality water suitable for human consumption.

In this study, we obtained acute toxicity data of 22 metals. The sensitivity correlation between our research and older studies shows that the hardness is a significant factor affecting metal toxicity and should be considered in determining reference EC_{50}s values. The efforts to establish this relationship between EC50 and physicochemical parameters have not been satisfactory, and some parameters could not explain the metal toxicity. Future studies should also evaluate the chronic toxicity and include other important metal exposure routes such as sediment and food. However, this work would be helpful in future studies that might lead to a universal theory of the effect of metal ion toxicity on aquatic organisms.

CONFLICT OF INTEREST

The Authors did not report any conflict of interest.

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