Aquatic acute toxicity assessments of molybdenum (+VI) to *Daphnia magna*

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Abstract

Generally, molybdenum (Mo) metals in the environment are very rare, but wastewater discharges from industrial processes may contain high concentrations of Mo, which has the potential to contaminate water or soil if not handled properly. In this study, the impact of three common compounds of hexavalent Mo (sodium molybdate (Na₂MoO₄·2H₂O), ammonium molybdate ((NH₄)₆Mo₇O₂₄·4H₂O) and molybdenum trioxide (MoO₃)) in an aquatic system were assessed based on 48-h exposure acute toxicity to *Daphnia magna* (*D. magna*). The LC₅₀ toxicities for associated conjugate ions including Na⁺, Cl⁻, SO₄²⁻, and NH₄⁺ were determined. Furthermore, the LC₅₀ values for the three forms of hexavalent Mo were determined, and the acute toxicities of the Mo forms were found to follow the order: (NH₄)₆Mo₇O₂₄·4H₂O > MoO₃ > Na₂MoO₄·2H₂O in solution. ([NH₄]₆Mo₇O₂₄·4H₂O exhibited the lowest LC₅₀ of 43.3 mg L⁻¹ (corresponding to 23.5 mg Mo L⁻¹) among the three molybdenum salts. The research confirmed that the toxicity of molybdenum in the aquatic system is highly dependent on the form of molybdenum salts used, and is also associated with the influence of the background water quality.

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(Huang et al., 2012). Among them, molybdate (+VI), an oxyanion with hexavalent Mo, is the most soluble Mo salt compound.

In the organism, Mo is one of the essential dietary micro-nutrients (Atia, 2008; Smalley et al., 2014), but excessive ingestion of Mo could result in anemia, gastrointestinal disturbances, hypothyroidism, bone and joint deformities, sterility, liver and kidney abnormalities, etc. (Shields, 2013; Diamantino et al., 2000). In view of this, the World Health Organization recommends a limit of 70 μg L\(^{-1}\) Mo in drinking water (WHO, 2011). Moreover, Taiwan, a high-tech industry export-oriented country, formulated the current regulatory concentration or standard for any controlled substance to be low compared to the LC\(_{50}\) in order to ensure that the toxic substance produces no observed adverse effects to the organism when exposure occurs in the environment. It has been reported that the concentration of Mo is low in natural environments, e.g., around 5 nM in river waters (Martin and Meyback, 1979). However, Mo in industrial wastewater could be high as 4–145 mg L\(^{-1}\) (Shan et al., 2012).

However, even though a maximum concentration level was regulated, several different Mo ions and/or compounds (e.g., different valences and associated conjugate cations or anions) in water may present different biological toxicities. For example, the median lethal concentrations (LC\(_{50}\)) for hexavalent Mo, sodium molybdate and ammonium molybdate, were reported to be 800 mg L\(^{-1}\) (rainbow trout, 96 h exposure) (Sigma-Aldrich, 2015a, b, c) and 420 mg L\(^{-1}\) (rainbow trout, 96 h exposure) (Sigma-Aldrich, 2015a, b, c), respectively, which are equivalent to 373 and 228 mg L\(^{-1}\) Mo. It can be seen that with the same test toxicity organism, ammonium molybdate presents a higher toxicity with respect to Mo than that induced by sodium molybdate. Hence, the toxicity of Mo in aquatic systems is also dependent on the contribution of its associated conjugate cations.

Bioassays, which rely on measuring the response of organisms exposed to contaminants, relative to a control, are the most widely used test methods for the toxicity assessment of chemical compounds and effluents. *Daphnia magna* (D. magna) (Fig. 1) is one of the biological organisms allowed in Taiwan's biological acute toxicity testing for the Water Pollution Control Act. *D. magna* is sensitive enough to distinguish between the toxicity to the different Mo compounds and is therefore a good test organism to predict the potential impact of new chemicals, or whole effluents, on the aquatic environment (Barmentlo et al., 2015; Stanley et al., 2013; Yim et al., 2006). This research was conducted to explore the impact of Mo (+VI) from different molybdate salts in aquatic system via *D. magna* acute toxicity bioassay. There are seven commercially available molybdenum salts, including ammonium molybdate, potassium molybdate, sodium molybdate, phosphor molybdcic acid, molybdenum disulphide, molybdenum trioxide, and molybdcic acid (Sajan overseas Ltd., 2015). Heijerick et al. (2012) indicated that the simple Mo\(_{12}\) is most likely to be formed from different molybdenum containing substances under common environmental conditions. The predominating ionic species of Mo (+VI) reported to be present in solution at pH > 2, were Mo\(_7\)\(_{14}\)\(\times\)\(_7\) (pH 2–7) and Mo\(_7\)\(_{12}\)\(\times\)\(_7\)\(_{1}\) (pH > 4) (Xiong et al., 2011). Molybdenum trioxide (Mo\(_3\)) is produced on the largest scale of any molybdenum compound (US Research Nanomaterials Inc., 2015). Therefore, Mo\(_7\)\(_{12}\)\(_{1}\) Mo\(_7\)\(_{12}\) and Mo\(_3\) were selected as reference test substances for the evaluation of Mo (+VI) compounds. As a first step, this study carried out a thorough LC\(_{50}\) evaluation of several aquatic impact factors including pH, conductivity, and background aquatic ions associated with Mo (+VI) salts. Thereafter, LC\(_{50}\) values for three different sources of molybdate (i.e., sodium molybdate (Na\(_2\)Mo\(_4\)), ammonium molybdate ((NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)\(\times\)\(_4\)), and molybdenum trioxide (Mo\(_3\)) were determined. Data from this study addresses the critical data gaps concerning the acute toxicity of Mo associated with releases to the environment from industrial processes.

## 2. Materials and methods

### 2.1. Chemicals

All reagents were of analytical grade and used without further purification. Sodium molybdate dihydrate (Na\(_2\)Mo\(_4\)•2H\(_2\)O, min 99%), ammonium molybdate tetrahydrate ((NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)•4H\(_2\)O, min 99%), molybdenum trioxide (Mo\(_3\), min 99%), sulfuric acid (H\(_2\)SO\(_4\), min 95%), potassium chloride (KCl, min 99.5%), sodium bicarbonate (NaHCO\(_3\), min 99.7%), sodium hydroxide (NaOH, min 99%), sodium chloride (NaCl, min 99.8%), nitric acid (HNO\(_3\), min 65%), ammonium hydroxide solution (NH\(_4\)OH, min 28%), ammonium sulfate ((NH\(_4\))\(_2\)SO\(_4\), min 99%) and sodium sulfate (Na\(_2\)SO\(_4\),...
of 25 minimum of once per day. A 16 h photo-period and a temperature
yeast powder and commercially available
(MgSO₄ 246 mg L⁻¹, NaHCO₃ 192 mg L⁻¹, CaSO₄⋅H₂O 120 mg L⁻¹ and KCl 8 mg L⁻¹), with a hardness of 170 ± 10 mg L⁻¹, and a dis-
solved oxygen (DO) concentration of greater than 5 mg L⁻¹. During
the period of cultivation, D. magna were fed on wheat germ powder;
yeast powder and commercially available fish feed mixture a
minimum of once per day. A 16 h photo-period and a temperature
of 25 ± 2 °C (controlled by the air conditioner), were maintained.

Furthermore, before carrying out the experiment, the test D. magna
were quarantined for 2 h without feeding. D. magna neonates
(younger than 24 h) were used in the toxicity test bioassay.

2.3. Experimental design

Initial experiments were designed to determine the LC₅₀ for
Na⁺, H⁺, NH₄⁺, SO₄²⁻ and OH⁻ as toxicity baselines in the aquatic
system. The concentration ranges associated with the toxicity
of these reagents were unknown prior to the experiment. Therefore,
several preliminary range-finding tests were conducted using
groups of 5 test organisms, which were exposed to several widely-
spaced sample dilutions to estimate the toxicity range (note that
these results exhibit no statistical significance and are not re-
ported.). Afterward, in the second step of the experiments, the
toxicities of different Mo sources (i.e., sodium molybdate, ammno-
ium molybdate, and molybdenum trioxide) were evaluated.

The 48 h acute toxicity tests were conducted in accordance with
the Taiwan NIEA method (NIEA, 2013). The tests were conducted
under static non-renewal conditions, using D. magna at 25 ± 2 °C
in a temperature controlled room, and a 16 h light and 8 h dark
photocycle were maintained. One set of experiments was con-
ducted using serial dilutions of the test solution (i.e., 20%, 40%, 60%,
80%, and 100%). Each concentration level test was carried out in
four replicates; each replicate was in a 40 mL vial (EPA volatile
organic compound sample vial) containing 25 mL of test solution
and five D. magna neonates of less than 24 h old, for a period of 48 h.
Each concentration level test was repeated three times. Therefore,
a total of sixty D. magna were used for one concentration level test.
Control tests in culture water (artificial hard water) were also
conducted side by side. No food was given during the course of all
experiments.

2.4. Analysis

The Mo ions concentration was determined using an inductively
coupled plasma atomic emission spectrometer (VARIAN 710 ES ICP-
OES, USA). A portable pH/ORP meter (TS-100, Suntex) equipped
with a pH electrode and a temperature probe was used to monitor
the solution pH and temperature in this study. DO and conductivity
were measured periodically using a portable DO meter (Hanna HI
9146, Woonsocket, RI, USA) and a pH/conductivity meter (Eutech
PC 5000, Singapore) equipped with a Fisher Scientific Accumet
four-cell conductivity probe, respectively.

3. Results and discussion

3.1. Background toxicity

The LC₅₀ values determined for the individual compounds
of NaCl, H₂SO₄, Na₂SO₄, NH₂OH, NaOH and (NH₄)₂SO₄ are presented in
Table 1 and the associated variations of pH, conductivity, and sur-
vival rate in different solutions during a 48 h period are shown in
Fig. 2. A reference toxicity test was conducted using the toxicant
NaCl to demonstrate the sensitivity of the test organisms prior to
performing further toxicity tests. The result of the NaCl reference
toxicant test indicated the LC₅₀ to be 3597 ± 248 mg L⁻¹ (61.6 mM)
(see Table 1), and the coefficient of variation (CV) obtained was
6.9%, which is within 50% of the recommended value by Taiwan
NIEA method (NIEA, 2013). Therefore, the acute toxicity test pro-
cedure used in this study exhibits a high reliability.

When comparing the pH and conductivities in NaCl test solu-
tions (see Fig. 2), pHs are similar at approximately neutral pH
conditions (pH 6.5–8.5), but the conductivities are much higher in
the 100% test solution than that in the 20% test solution (e.g.,
15,500 µS cm⁻¹ for 143.30 mM (100%) NaCl solution versus
4000 µS cm⁻¹ for 29.06 mM (20%) NaCl solution). There was 100%
mortality of the D. magna in the 143.30 mM NaCl test solution, and
the survival rate of D. magna was 72% in the 29.06 mM NaCl test
solution. Note that a survival rate of 100% was observed in the blank
control solution with the conductivity of 1000 µS cm⁻¹. Therefore, it
can be preliminarily concluded that a near neutral pH (6.5–8.5) and
a conductivity of less than 4000 µS cm⁻¹ might not cause D. magna
mortality.

Moreover, the effects of H⁺ and OH⁻ on D. magna survival were
evaluated to determine their LC₅₀ concentrations. The LC₅₀ for H⁺
was 0.24 ± 0.04 mg L⁻¹ (0.12 mM carried out using H₂SO₄). The LC₅₀
concentrations for OH⁻ were determined using NH₄OH and NaOH,
and were found to be 12.9 ± 1.5 mg L⁻¹ (0.76 mM using NH₄OH) and
53.3 ± 3.8 mg L⁻¹ (3.14 mM using NaOH) (see Table 1). Note that
the LC₅₀ values of Na₂SO₄ (18.5 ± 2.8 mg L⁻¹ (0.13 mM)) and (NH₄)₂SO₄
(26.6 ± 3.2 mg L⁻¹ (0.76 mM)) were evaluated as reference toxic-
ities for H₂SO₄, NH₂OH, and NaOH. The LC₅₀ for Na₂SO₄ is associated
with Na⁺ concentration of 5.9 mg L⁻¹ and a SO₄²⁻ concentration
of 12.3 mg L⁻¹ (pH ~7.5). The LC₅₀ for NaCl is associated with a Na⁺
concentration of 1416 mg L⁻¹, which is much higher than the
5.9 mg L⁻¹ determined from the Na₂SO₄ test. Therefore, it can be
assumed that the Na⁺ concentration did not cause the toxicity in
the Na₂SO₄ test, and that the toxicity was the result of the SO₄²⁻
concentration. Further evaluation of the results of the H₂SO₄ test
found the corresponding LC₅₀ for SO₄²⁻ to be 11.6 mg L⁻¹. Based on
the similar LC₅₀ for SO₄²⁻ obtained from H₂SO₄ and Na₂SO₄ tests, it
can be deduced that the SO₄²⁻ concentration of approximately
12 mg L⁻¹ may be acutely toxic to D. magna. Nevertheless, even
though pH in the H₂SO₄ solution was at near neutral condition
(except for the 0.2 mM solution (100%), which had a pH of 4), the
H⁺ in the solution may have added to the toxicity of SO₄²⁻. It should
be noted that the conductivity of the solution (<600 µS cm⁻¹) was
much smaller than the 4000 µS cm⁻¹ conductivity measured in the
NaCl test, and therefore conductivity in this test likely did not
contribute to mortality in any significant measure.

The LC₅₀ for NaOH is associated with a Na⁺ concentration
of 72.1 mg L⁻¹ and OH⁻ of 53.3 mg L⁻¹. The Na⁺ concentration is much
less than that determined in the NaCl test and therefore would not
be responsible for acute toxicity in the NaOH solutions. Therefore,
the D. magna mortality is perhaps due to the increased OH⁻ ion
concentration (e.g., pH ~11 for 5.0 mM NaOH (100%) and pH ~10.5 for 4.0 mM NaOH (80%)). Furthermore, the LC50 for NH4OH corresponds to an NH4\(^+\) concentration of 13.6 mg L\(^{-1}\)/C0\(^-1\) and OH\(^-\)/C0\(^-1\) of 12.9 mg L\(^{-1}\)/C0\(^-1\) (pH ~ 9.5 to 10.0). Although the concentration of OH\(^-\)/C0\(^-1\) in the NH4OH solution was lower than that in the NaOH solution, the pH in all dilutions of the NH4OH solution exceeded the pH safety threshold (pH = 6.5–8.5). Based on this, it appears that the simultaneous presence of both NH4\(^+\) and OH\(^-\) may result in greater toxicity than each individual ion. On the other hand, the results of the (NH4)\(_2\)SO4 test indicated that the LC50 of (NH4)\(_2\)SO4 is associated with an NH4\(^+\) concentration of 11.0 mg L\(^{-1}\)/C0\(^-1\) and SO4\(^2-\)/C0\(^-1\) of 11.7 mg L\(^{-1}\). The NH4\(^+\) value from the (NH4)\(_2\)SO4 test was comparable to that from the NH4OH test (NH4\(_2\) LC50 = 13.6 mg L\(^{-1}\)), and the SO4\(^2-\) value from the (NH4)\(_2\)SO4 test was also similar to that from the Na2SO4 test (SO4\(^2-\)/C0\(^-1\) LC50 = 12.3 mg L\(^{-1}\)). Moreover, the trend of survival rates from the different diluted solutions was similar to the NH4OH and Na2SO4 tests. Hence, in addition to the previous threshold levels determined for pH and conductivity, it can be further concluded that NH4\(_2\) (11 mg L\(^{-1}\)) and SO4\(^2-\) (12 mg L\(^{-1}\)) may pose toxicity to D. magna in water bodies.

3.2. Toxicity of Mo ion (+VI) and associated salts in the aquatic system

Different sources of Mo ions (e.g., different valences or bonded with conjugate cations or anions) in water may result in different biological toxicities. Therefore, various species of Mo salts (i.e., Na2MoO4•2H2O, (NH4)\(_6\)Mo7O24•4H2O and MoO3) were tested for their biological acute toxicities. The variations of pH and conductivity, and the resulting survival rate of D. magna in solution during a 48 h period, are shown in Fig. 3. From the bioassay test using D. magna, the LC50 values were 367.8, 43.3 and 89.2 mg L\(^{-1}\) for Na2MoO4•2H2O, (NH4)\(_6\)Mo7O24•4H2O and MoO3, respectively (Fig. 4). In the Na2MoO4•2H2O test, the LC50 is associated with a Na\(^+\) Table 1

<table>
<thead>
<tr>
<th>Species Initial Conc.</th>
<th>LC50 (mM)</th>
<th>mg L(^{-1})/C0(^-1) (species)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl 145.30</td>
<td>61.6 ± 4.3</td>
<td>(NaCl) 3597 ± 248 (Na(^+)) (Cl(^-))</td>
</tr>
<tr>
<td>H2SO4 0.20</td>
<td>0.12 ± 0.02</td>
<td>(H2SO4) (H(^+)) (SO4(^2-))</td>
</tr>
<tr>
<td>Na2SO4 0.20</td>
<td>0.13 ± 0.02</td>
<td>(Na2SO4) (Na(^+)) (SO4(^2-))</td>
</tr>
<tr>
<td>NH4OH 1.02</td>
<td>0.76 ± 0.09</td>
<td>(NH4OH) (NH(_4)) (OH(^-))</td>
</tr>
<tr>
<td>NaOH 5.00</td>
<td>3.14 ± 0.22</td>
<td>(NaOH) (Na(^+)) (OH(^-))</td>
</tr>
<tr>
<td>(NH4)(_2)SO4 0.50</td>
<td>0.31 ± 0.004</td>
<td>((NH4)(_2)SO4) (NH4(_2)) (SO4(^2-))</td>
</tr>
</tbody>
</table>

Fig. 2. Variations of pH, conductivity, and survival rate in (a) NaCl, (b) H2SO4, (c) Na2SO4, (d) NH4OH, (e) NaOH, and (f) (NH4)\(_2\)SO4 solutions of acute toxicity tests.
concentration of 35.01 mg L$^{-1}$ and Mo$^{6+}$ of 145.8 mg L$^{-1}$ (pH = 7.5–8.5). Additionally, the LC$_{50}$ of (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O is associated with a NH$_4^+$ concentration of 3.8 mg L$^{-1}$ and Mo$^{6+}$ of 23.5 mg L$^{-1}$ (pH = 7.0–8.0). It can be seen that both the LC$_{50}$ of Na$^+$ and NH$_4^+$, and pH and conductivity in the Na$_2$MoO$_4$·2H$_2$O and (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O tests are within the threshold levels or much lower than those reference toxicity levels determined earlier. Therefore, the MoO$_3^{2-}$ LC$_{50}$ concentration of 243.1 mg L$^{-1}$ and Mo$_7$O$_{24}^{6-}$ LC$_{50}$ concentration of 37.0 mg L$^{-1}$ appear to indicate that these compounds can be acutely toxic to _D. magna_.

Molybdenum trioxide, in the form of the rare mineral molybdate, dissolves slightly in water (e.g., 1.0 g MoO$_3$ L$^{-1}$ at 20 °C).

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Fig. 3. Variations of pH, conductivity, and survival rate in (a) Na$_2$MoO$_4$·2H$_2$O, (b) (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, and (c) MoO$_3$ solutions of acute toxicity tests.

Fig. 4. The LC$_{50}$ values of Mo$^{6+}$ obtained from different salts: Na$_2$MoO$_4$·2H$_2$O, (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O, and MoO$_3$. 

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(Sigma-Aldrich, 2015a, b, c). However, the dissolved MoO3 would react with OH\(^-\) to form H\(^-\)O\(\cdot\)MoO\(_3\) and subsequently MoO\(_2\)\(^5-\) (Eqs. (1) and (2)), thereby resulting in the decrease of solution pH (Krishnan et al., 2009). Hence, gradual decreases in pH were observed when the MoO\(_3\) concentration increased (see Fig. 3).

\[
\text{MoO}_3 + \text{OH}^- \rightarrow \text{H}^- \cdot \text{MoO}_3 \\
\text{OH}^- + \text{H}^- \cdot \text{MoO}_3 \rightarrow \text{MoO}_2^{5-} + \text{H}_2\text{O}
\]

(1) and (2)

The LC\(_{50}\) for the MoO\(_3\) is associated with Mo concentration of 59.5 mg L\(^{-1}\) and pH is in the range of 3.0–8.0. The \(D.\, m\_g\_na\_\) mortality in all of the diluted solutions exceeded 70% except for the 20% diluted solution, in which the pH was approximately 8.0. Therefore, it can be deduced that the presence of Mo at acidic pH caused the \(D.\, m\_g\_na\_\) mortality. Note that aqueous conductivity is far less than the toxicity threshold level. After the toxicity test, the concentration of Mo ions was measured using an ICP-AES and the results indicated that Mo in all of the test solutions did not significantly change during the bioassays (i.e., \(\pm 0.1\%\) (data not shown)). This may indicate that Mo was not significantly absorbed by the \(D.\, m\_g\_na\_\) during the test.

4. Conclusion

According to the results obtained in this study, the acute toxicity for different Mo compounds within a 48 h exposure increased in the following order: \(\text{Na}_2\text{MoO}_4\cdot2\text{H}_2\text{O} < \text{MoO}_3 < (\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot4\text{H}_2\text{O}\) in solutions. The data showed that MoO\(_2\)\(^5-\) presents the most toxic dissolved species (i.e., with the lowest LC\(_{50}\) of 37.0 mg L\(^{-1}\) among the three molybdenum salts). In terms of acute toxicity caused by Mo alone, the use of \((\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot4\text{H}_2\text{O}\) reveals the lowest LC\(_{50}\) of 23.5 mg Mo L\(^{-1}\) and the \(\text{Na}_2\text{MoO}_4\cdot2\text{H}_2\text{O}\) is the least toxic substance. The pH and conductivity may also contribute biological toxicity and hence should be limited to pH in the range of 6.5–8.5 and a conductivity of less than 4000 \(\mu\)S cm\(^{-1}\). As for the effects of Mo salts and associated conjugate cations or anions, it can be suggested that the concentration of \(\text{NH}_4\) of <11 mg L\(^{-1}\), \(\text{SO}_4\)\(^2-\) of <12 mg L\(^{-1}\) and \(\text{Na}^+\) of <1416 mg L\(^{-1}\) be considered when evaluating the potential toxicity to \(D.\, m\_g\_na\_\) aquatic systems. The data showed that the level of molybdenum toxicity in aquatic systems is highly dependent on the form of molybdenum salts used and also on the background water quality and associated conjugate ion species.

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References