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TOXICITY OF SOME INORGANIC POTASSIUM SALTS TOWARDS DUCKWEED *(LEMNA MINOR* L.*)*

Donyo H. Ganchev

Agricultural University – Plovdiv, Bulgaria
E-mail: d_ganchev@au-plovdiv.bg

Abstract

The toxicity of six potassium inorganic salts: potassium carbonate (K_2CO_3), potassium hydrogen carbonate ($KHCO_3$), potassium sulfate (K_2SO_4), dipotassium hydrogen orthophosphate (K_2HPO_4), monopotassium phosphate (KH_2PO_4) and potassium aluminum sulfate ($KAl(SO_4)_2.12H_2O$) towards duckweed (*Lemna minor* L.) was studied. The results showed that all tested chemicals were with low toxicity but similar salts as potassium carbonate and potassium hydrogen carbonate or dipotassium hydrogen orthophosphate and monopotassium phosphate can express different toxic action towards the tested object. Potassium aluminum sulfate, was toxic to duckweed at very narrow ranges: up to 0.8 % concentration but the salt did not cause any damage to the plants. At 1 % concentration, the first phytotoxic symptoms appeared and at 1.44% concentration LC50 was established and LC90 was reached at concentration of 1.65%. The most toxic chemical was potassium carbonate due to the higher pH of its solutions, but KH_2PO_4 was found to be more toxic than K_2HPO_4 although its solutions were with neutral pH, while a pH of solution of K_2HPO_4 over 1 % concentration was slightly alkaline.

Keywords: *Lemna minor* L., potassium carbonate, potassium hydrogen carbonate, potassium sulfate, dipotassium hydrogen orthophosphate, monopotassium phosphate, potassium aluminum sulfate

INTRODUCTION

Duckweed (*Lemna minor* L.) is a standard ecotoxicological object widely used in experiments for revealing the possible toxicity of chemicals towards aquatic ecosystems. The plant is distributed almost all over the world, it is easy to be grown and is sensitive to the chemical pollution of water (Radić et al., 2010; Pop et al., 2021). *Lemna minor* L. is also a classically used aquatic plant for heavy metals removing and bioremediation (Axtell et al., 2003; Ozengin & Elmaci, 2007; Ekperusi et al., 2019).

In the present research several potassium inorganic salts were examined for their possible toxicity toward *Lemna minor* L. Selected salts are common chemicals used both in chemical and food industry but can be used also in the agriculture as low toxic, cheap and easy to be used pesticides. However, up to this moment there is no information how these chemicals affect duckweed. There is information about their general aquatic toxicity and nothing specific about *Lemna minor* L. Such investigation is important for reveling their toxicity in this aspect for their safe application in the agriculture, especially, organic agriculture and integrated pest management as well as improving QSAR analysis of this chemicals (Freidig et al., 2007; Ambure & . Cordeiro, 2020).

Potassium carbonate (K_2CO_3) is an inorganic salt, with alkaline properties. The salt is used widely in soap manufacturing, as a fire suppressant in extinguishing deep-fat fryers, in the welding fluxes, in the food industry for alkalization of cocoa powder, noodles, ramen; as a buffering agent in the production of mead or wine. It is used also in animal husbandry as a source of potassium for animals (Naser et al., 2013). Some studies show the potential of potassium carbonate for a biodiesel production (Baroi et al., 2009; Duan et al., 2013). Potassium carbonate however, can express skin and eye damage or irritation, and STOT SE 3 (specific target organ toxicity, single exposition, H371, signal word "Warning") towards respiratory tract irritation. Water solubility is very high = 1120 g/l at 20°C. LD50 acute toxicity towards rat is over 2000 ppm (oral or dermal), towards fishes is 68 ppm and towards aquatic invertebrates is 200 ppm (Mumford et al., 2012; Gohla et al., 2021). The salt has also antifungal effect towards *Botrytis cinerea* and *Fusarium solani* (Ghadiri et al., 2013; Arslan et al., 2009; Zaker, 2014; Türkkan et al., 2017).

Potassium hydrogen carbonate or potassium bicarbonate ($KHCO_3$) is manufactured by treating an aqueous solution of potassium carbonate with carbon dioxide. The salt is an alternative of baking soda (sodium bicarbonate). Other uses include pH regulation, a buffering agent in various medicals, and as an additive in winemaking (He & MacGregor, 2001). The salt has application in medicine as a control agent of calcium excretion and improves calcium balance, as well as urinary nitrogen excretion (Frassetto et al., 2005; Dawson-Hughes et al., 2009). Potassium hydrogen carbonate is also widely used in fire extinguishers as a fire suppression agent (Hisatsune & Adl, 1970; Kuang et al., 2011). In the pest management it is used also as fungicide against powdery mildew and apple scab, cercospora leaf spot on sugar beet, *Botrytis cinerea*, *Sclerotinia sclerotiorum* (Ordóñez-Valencia et al., 2009; Marku et al., 2014; Laurent et al., 2021; 1Sehsah et al., 2022). The salts also reduce postharvest decay on bell pepper fruits (Fallir et al., 1997). Potassium hydrogen carbonate has very low toxicity towards humans and environment. LD50 oral / dermal rat is over 2000 ppm; LC50 acute toxicity for fish is 1300 ppm, for *Daphnia manga* - 360 ppm. Its water solubility is 362 g/l at 25°C.

Dipotassium hydrogen orthophosphate (K_2HPO_4) is a traditional food additive used as emulsifier and stabilizer or buffering and chelating agent in the dairy products. In the agriculture can be used as fertilizer (Davarpanah et al., 2014; Baiea et al., 2015). The salt is non-toxic for humans, animals or the aquatic environment. LD50 acute toxicity towards rat is over 5000 ppm oral or dermal. Its water solubility is very high -1160 g/l at 20°C.

Dipotassium hydrogen orthophosphate together with monopotassium phosphate were found to have antifungal activity towards plant pathogenic fungi of *Sclerotinia sclerotiorum, Puccinia triticina and Uromyces appendiculatus* (Arslan, 2015) as well as against *Alternaria solani* causing early blight disease of tomato (Imran et al., 2022).

Monopotassium phosphate (K_2HPO_4) is similar to dipotassium phosphate inorganic salt with almost the same applications and crystalline structure. The salt is used also in the sports drinks such as Gatorade© and Powerade© and in medicine as phosphate substitution in hypophosphatemia (Sajyan et al., 2018). However, the water solubility of monopotassium phosphate is lower than dipotassium phosphate - 208 g/l at 20°C. LD50 acute toxicity towards rat is also lower than dipotassium phosphate - over 2000 ppm (oral / dermal), however the salt is still classified as low toxic. Towards acute toxicity of fishes, its LC50 is over 100 ppm and towards daphnia and other aquatic invertebrates LC50 is also over 100 ppm. Its toxicity towards algae (*Desmodesmus subspicatus*) is also over 100 ppm (Verrett et al., 1980; Weiner et al., 2001). The salt, just like dipotassium phosphate, can have antifungal effect on plant pathogenic fungi (Reuveni & Reuveni, 1998; Napier & Oosthuyse, 2000).

Potassium sulfate (K_2SO_4) is an inorganic salt predominantly used as a fertilizer (Mona et al., 2011), but also in the glass production and as an agent in the pyrotechnics (Bring et al., 2006). Water solubility is 110 g/l at 20°C. Potassium sulfate has a very low toxicity towards humans and environment - LD50 oral /dermal acute toxicity for rat is over 2000 ppm, LC50 acute toxicity for fish is 680 ppm, for aquatic invertebrates – 720 ppm, for algae – 2900 ppm (Carl Roth., 2023)

Potassium aluminum sulfate (potassium alum) with chemical formulae $KAl(SO_4)_2.12H_2O$ is an inorganic salt typically used in the leather tanning, water purification, in the deodorants and in the medicine as bleed stopping agent (Young, 2004; Freitas et al., 2016). The salt has antimicrobial properties too. In cosmetic and medicine is widely used against acne, ulcers, canker sores, gingival inflammations (Mirjalili & Karimi, 2013; Pinheiro et al., 2017). The salt was proved to have pesticide properties against fire blight (Reininger et al., 2017; Gaganidze et al., 2021) and as antimycotic pesticide in post – harvest treatments of tomatoes and other fruits (Amadi et al., 2019). Potassium aluminum sulfate have 139 g /l at 20 °C water solubility and is not be classified as acutely toxic. LD50 for rat oral is over 2000 ppm. Also is with low toxicity for the environment: LC50 acute toxicity for fish is 100 ppm; for aquatic invertebrates – 960 ppm (Carl Roth, 2022).

The aim of present study is to examine aquatic toxicity of selected inorganic potassium salts towards duckweed (*Lemna minor* L.).

MATERIALS AND METHODS

Fronds from duckweed (*Lemna minor L.*) naturally inhabited river "Nevolja" in village Vasil Levski, Plovdiv district, Bulgaria, were used in the research. The inorganic salts used in the study were:

- K_2CO_3: CAS Number - 584-08-7; Purity 99 %, supplied by Research Products International Corp.
- $KHCO_3$ - CAS Number - 298-14-6; Purity 99 % supplied by Eisen- Golden Laboratories.
- K_2SO_4 – CAS Number 7778-80-5; Purity 99.9 %, supplied by Zeus Chemicals & Exporters.
- K_2HPO_4 - CAS Number 7758-11-4; Purity 98% supplied by Research Products International Corp.
- KH_2PO_4: CAS Number 7778-77-0; Purity 99 % supplied by Eisen- Golden Laboratories.
- $KAl(SO_4)_2.12H_2O$: CAS Number 7784-24-9; Purity 99.7 %, supplied by Eisen- Golden Laboratories.

According to the OECD Guideline, the salts were tested in ten concentrations (at least five according to the Guideline) arranged in a geometric series with separation factor 3. Plastic cups with 200 ml volume were used, and in each cup 150 ml water solution (distilled water) of tested salt at the given concentration was placed. The test salts concentrations were maintained during the experiment by dilution of distilled water (for elimination of the influence of any other chemicals on toxicity of tested salts towards duckweed) when the volume level of solutions dropped below 150 ml.

Each test variant consisted of 5 plastic cups (replicates). The number of fronds in each test vessel was equal to ten. The test vessels were placed at the room temperature - 24°C A randomized design for location of the test vessels was applied for minimizing the influence of spatial differences in light intensity or temperature. The tests were visually examined 7 days after the plants were transferred into the test vessels and frond numbers appearing normal or abnormal were determined every 3 days from the beginning of the test.

Visual changes in plants such as necrosis, chlorosis or gibbosity, colony break-up or whitening were observed (Alkimin et al., 2019; Loll et al., 2022) and the percent of plant fronds with such sings was determined in each test vessel. On the base of these data a Dose – Response Modeling was performed via R language for Statistical Computing and drc packge (Ritz et al., 2016) and NOAEC (No Adverse Effect Concentration), LOAEC (Lowest Adverse Effect Concentration), LC50 (Lethal Concentration – 50 %) and LC90 (Lethal Concentration – 90 %) were also determined. One-Way ANOVA analysis was performed via R Language for establishing statistical significant differences between tested variants (Ihaka & Gentleman, 1996, Davies et al., 2003; Yu, 2006, Crawley, 2012, Wilson & Koch, 2013).

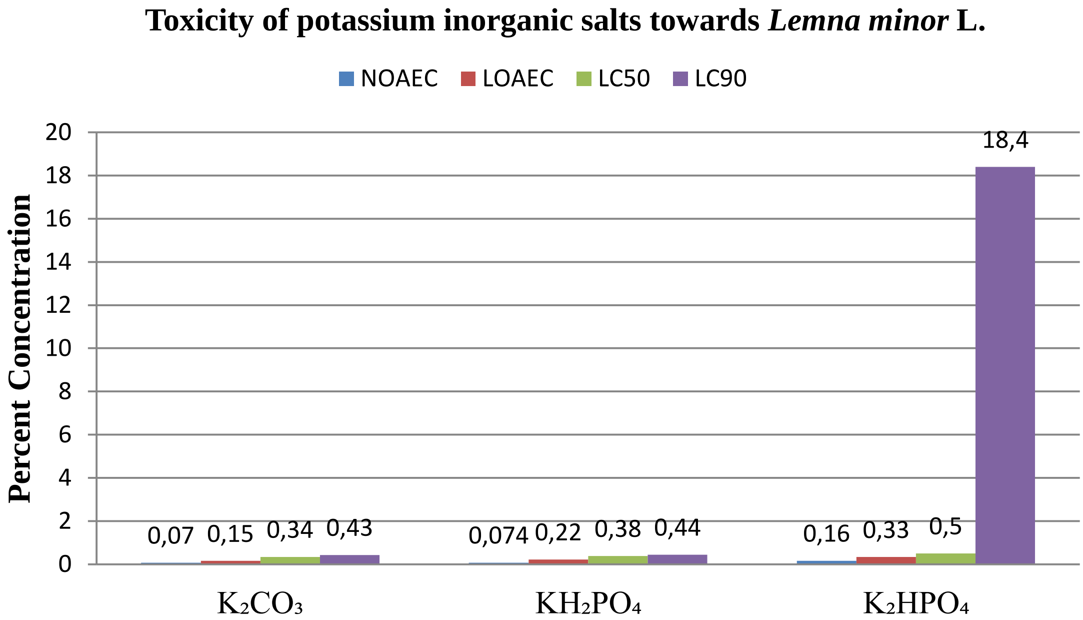

Figure 1. Toxicity of K_2CO_3, KH_2PO_4 and K_2HPO_4 towards *Lemna minor* L.

RESULTS

In the Figure 1 are presented results about experimental variants with potassium carbonate, dipotassium hydrogen orthophosphate and monopotassium phosphate towards duckweed.

The data showed, a very low toxicity of monopotassium phosphate – the established LC50 was 0.5 % concentration (5000 ppm) and LC90 – 18.4 %. Up to the 0.16 % (1600 ppm) the salt did not cause any damages to plants. However, the results clearly showed the differences in toxicity towards *Lemna minor* L. of monopotassium phosphate and its similar salt dipotassium hydrogen orthophosphate, although pH of their solutions was the same – between 7and 8 ($p<0.05$). Dipotassium hydrogen orthophosphate expressed much higher toxicity than monopotassium phosphate – the established LC50 was 0.38 % (3800 ppm), LC 90 = 0.44 % (4400 ppm). Up to the 740 ppm the salt did not cause any damage to plants. Potassium carbonate caused higher toxicity towards tested aquatic plants, probably due to the high pH of its solutions. Up to 0.01 % pH was 7; at 0.1 % concentration – 9 and over 1 % concentration – 14. Up to 0.07 % (700 ppm), pH = 8; the salt did not cause any damages on tested plants. Established LC 50 was 0.34 (3400 ppm), pH of solutions =14.

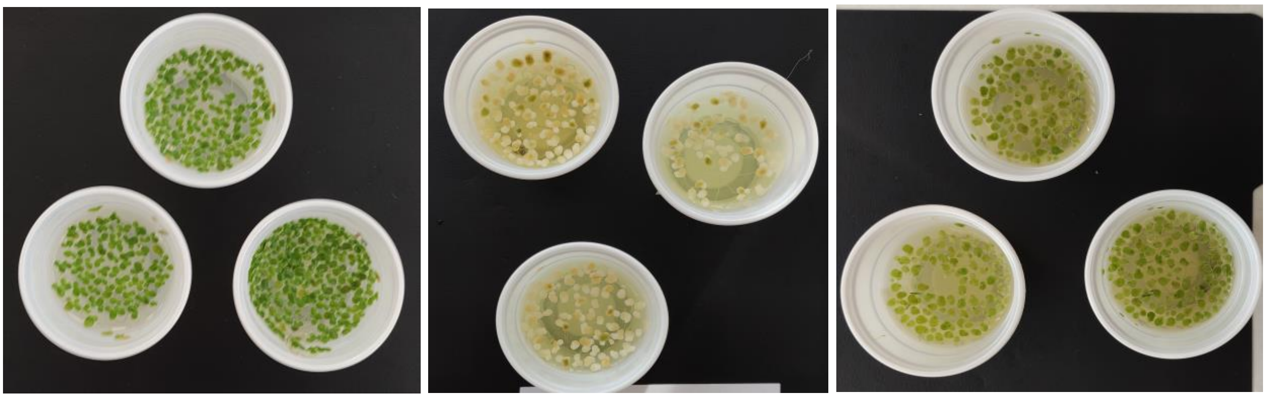

Figure 2. In the left: control variant; in the middle: K2CO3 in 0.5 % concentration; in the right: K2HPO4 in 0.4 % concentration

The Figure 3 shows the results from tests with potassium hydrogen carbonate, potassium sulfate and potassium aluminum sulfate

The results revealed that potassium hydrogen carbonate was less toxic toward duckweed in comparison with the similar salt potassium carbonate which could be due to the lowest pH of its solutions. Potassium sulfate in comparison with potassium aluminum sulfate, has significantly lower LC50 but the full destruction of the plants was observed at over 7.5 % concentration, while potassium aluminum sulfate was able fully to devastate the *Lemna minor* L. plants at only 2 % concentration. The conducted tests reveal that potassium aluminum sulfate expresses its toxicity towards duckweed at very narrow ranges, the salt did not cause any damage to the plants up to 0.8 % concentration, at 1 % concentration, the first phytotoxic symptoms appeared, at 1.44 % concentration LC50 was established and at 1.65 % concentration – LC90.

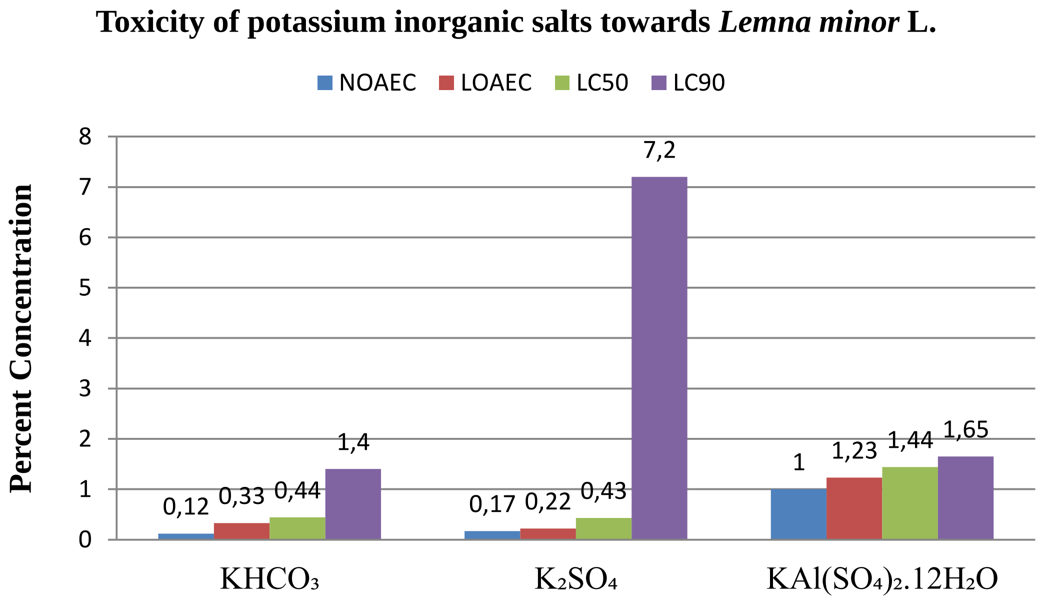

Figure 3. Toxicity of KHCO3, K2SO4 and KAL(SO4)2 towards Lemna minor L.

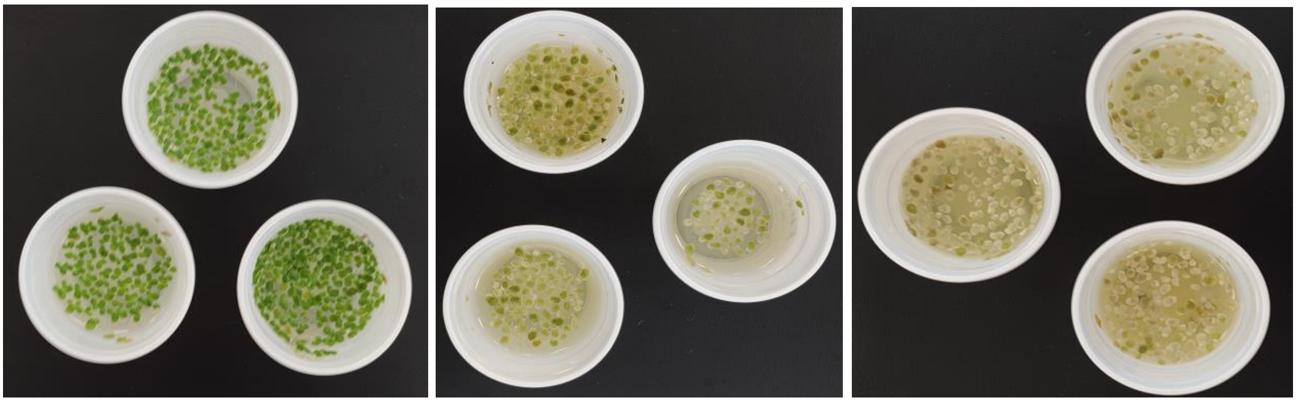

Figure 4. In the left: control variant; in the middle: KHCO3 in 0.5 % concentration; in the right: KAL(SO4)2 in 2 % concentration

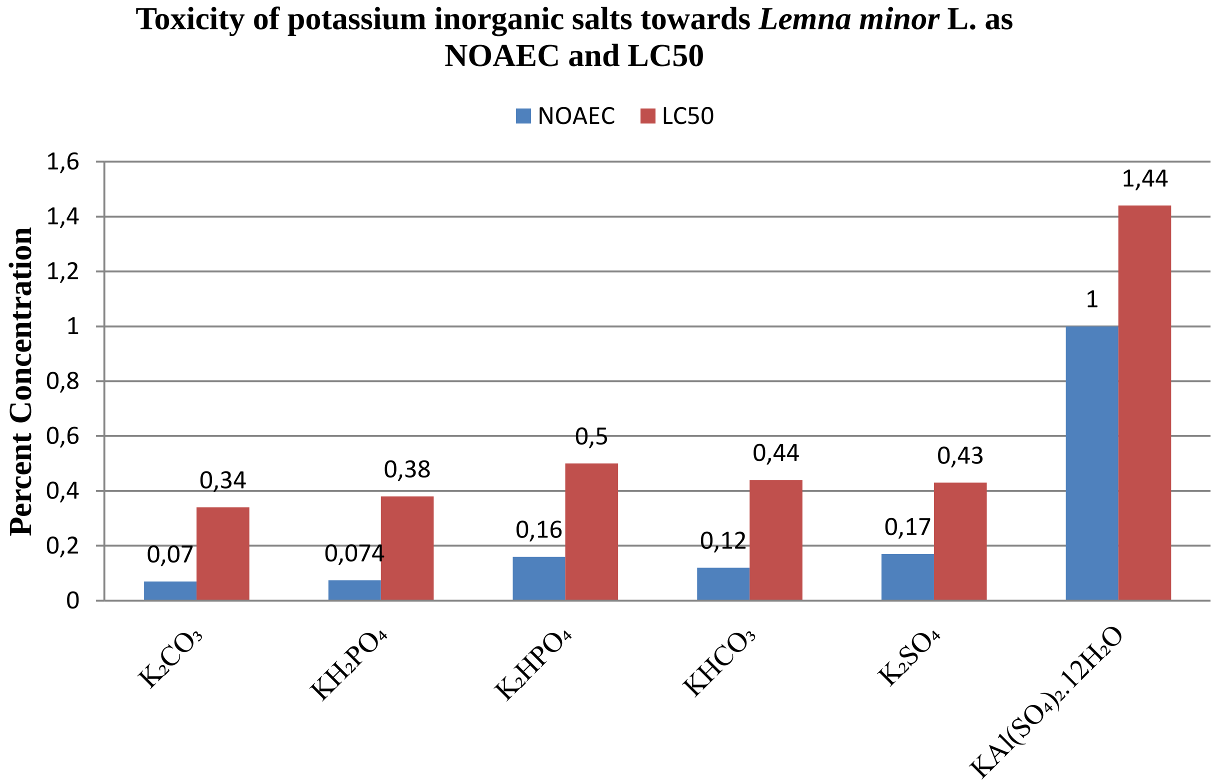

Figure 5. NOAEC and LC50 for K2CO3, KH2PO4, K2HPO4, KHCO3, K2SO4 and KAL(SO4)2 towards Lemna minor L.

The Figure. 5 shows summarized results expressed as NOAEC and LC50 for all chemicals in conducted tests with toxicity towards *Lemna minor* L.

The results clearly showed that potassium aluminum sulfate was less toxic according to the NOAEC and LD50 towards tested duckweed.

According to LC50, other tested inorganic salts have a similar toxicity, however towards the NOAEC, K_2CO_3 and KH_2PO_4 expressed higher toxicity than others, while according to the same indicator (NOAEC), the toxicity of K_2HPO_4, $KHCO_3$ and K_2SO_4 was similar.

DISCUSSION

E ven through, the potassium carbonate is considered as non-toxic chemical including towards aquatic organisms (Gohla et al., 2021) the present study showed that it can express toxicity to duckweed in 3400 ppm. The same effect was found for potassium hydrogen carbonate which is also considered as low risk chemical towards aquatic plants and algae (Alvarez et al., 2021) and the trials reveal that its toxicity is even lower than potassium carbonate. ANOVA analysis reveal that there were statistically proven differences ($p<0.05$) in the toxicity toward duckweed between potassium carbonate and potassium hydrogen carbonate. Dipotassium hydrogen orthophosphate, monopotassium phosphate and potassium sulfate expressed the same (very low) toxicity towards *Lemna minor* L. The conducted ANOVA analysis showed no significant statistical differences between them ($p>0.05$) which is similar to the oral acute toxicity of monopotassium phosphate and potassium sulfate towards rat. Potassium aluminum sulfate was the less toxic chemical from all tested in the given research (statistically proven differences ($p<0.05$) which imply for its toxicological safety (National Center for Biotechnology Information, 2023). Conducted Dose – Response Modeling (aka regression analysis) shows classical S-shaped Dose – Response Curve for K_2CO_3, very similar to the L-shaped curves for K_2HPO_4 and K_2SO_4. The shape of the curve of $KHCO_3$ was also similar to the curves of K_2HPO_4 and K_2SO_4. However, KH_2PO_4 and especially $KAl(SO_4)_2$ showed very unspecific dose response curves as linear straight curve.

CONCLUSION

The conducted trials showed that all tested inorganic potassium salts (potassium carbonate, potassium hydrogen carbonate, potassium sulfate, dipotassium hydrogen orthophosphate, monopotassium phosphate and potassium aluminum sulfate have a low toxicity towards duckweed (*Lemna minor L.*). However, similar salts as potassium carbonate and potassium hydrogen carbonate or dipotassium hydrogen orthophosphate and monopotassium phosphate can express different toxic actions towards the tested objects. Potassium sulfate, although it has LC50 = 0.43 %, can fully destroy the plants at very high concentrations (LC90= 7.2 %). The most toxic chemical from the tested salts was potassium carbonate due to the higher pH of its solutions, but KH_2PO_4 was found to be more toxic than K_2HPO_4 although its solutions were with neutral pH, while pH of the solution of K_2HPO_4 over 1 % concentration was slightly alkaline. The presented study showed that potassium carbonate, potassium hydrogen carbonate, potassium sulfate, dipotassium hydrogen orthophosphate, monopotassium phosphate and potassium aluminum sulfate can be applied safely as industrial or agricultural chemicals close to the aquatic ecosystems in the aspect of their established in this study low toxicity to aquatic higher plant confirming the known data up to this moment, for their very low aquatic toxicity.

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