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Toxicity Evaluation of Different Exposure Scenarios of Road Dust Using *Daphnia magna* and *Artemia salina* as Aquatic Organisms, and *Prosopis cineraria* and *Vachellia tortilis* as Native Plant Species

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Abstract

Particulate matter (PM10) deposited as road dust is considered an important source of contamination from atmosphere. However, there are limited studies on the toxicity of road dust as such on different organisms. This study evaluates the toxicity of road dust using different extraction scenarios on *Daphnia magna* and *Artemia salina* as aquatic organisms and also on *Prosopis cineraria* and *Vachellia tortilis* as local plant species. Chemical analysis of different extracts shows considerable amount of trace metals, however the trace metals in the dust extract associated with suspended sediment were not absorbed by the receptors. On the other hand, the concentration of trace metals in the artificial mixture was found bioavailable and absorbed causing a high percentage of mortality. In the plant assay, significant difference was obtained in the germination percentage between the control and three different extraction exposures in both plant species. The mean root length of *P. cineraria* and *V. tortilis* were higher in 20% and 50% extracts than the control probably due to the availability of nutrients from the dust extract. Interestingly however, the seedling vigor index was the opposite with higher index in the control and lower in dust extracts that contain heavy metals.

Keywords

Road Dust, Heavy Metals, Toxicity, Bioavailability, Holding Time

1. Introduction

In urban environments with high population density and enormous anthropogenic activities, road dust is considered as one of the significant nonpoint sources of pollution. Road dust is composed of complex particulates such as organic compounds, heavy metals, other inorganic substances, mold spores and pollen which get suspended in the atmosphere due to movement of vehicles and wind. Studies have shown that anthropogenic sources of potential toxic heavy metal emissions are mainly due to traffic emissions [1], especially exhaust and non-exhaust processes [2].

Road dust is a valuable medium of characterizing urban environmental quality and may act as temporary source of pollutants including heavy metals from a variety of sources leading to atmospheric pollution through re-suspension [3] [4]. There are several sources of these pollutants such as vehicular movement [5], road surface wear and tear, brake pad corrosion, brake oil leakage, road paint, abrasion of tires, etc. Dust contaminated by heavy metals is becoming an important threat to urban environment because metals in the dust can easily be transferred into human body by ingestion, inhalation and dermal contact [6] [7]. Road dust is considered a significant source of sediment contamination and is often flushed into the surface waters through road runoff [8].

The main exposure pathways of heavy metals to aquatic organisms are through dissolved phase and direct contact with contaminated particles [9]. Biotoxicity evaluation depends on subjecting test organisms to all the bioavailable chemicals in the exposure test and subsequently noting the changes in biological activity [10]. Sediment bioassays have been studied widely [8] [9] [11]-[14]. Organisms' choice for a biotoxicity test depends on the chemicals exist in the sample, the prediction to be made, the time needed, and cost involved to perform a test [15].

In the terrestrial environment, soil is an essential source and serves as a primary substrate necessary for seed germination, survival and growth of plants [16]. Though trace metals in soil are essential for development and growth of plants, soil ecosystems are polluted with heavy metals by human-induced activities [17]. Essential and nonessential metals, when exceeding the threshold limits, can cause different problems such as growth inhibition, mutagenic effects, and increased mortality [18]. Higher metal concentrations might interfere with the inhibition of several plant physiological processes and development: including photosynthesis, mineral nutrition, sugar transport, seedling growth, and seed germination [19].

Seed germination is the most sensitive physiological process among all stages in plants' life cycle [20]. Heavy metals' effect on seed germination has been investigated by several studies regarding its high sensitivity to metal pollution [20] [21]. It was reported that the effect of heavy metals on seed germination is associated with the type of species, the nature and the content of trace metals [22]. In this study we evaluated the toxicity of road dust collected from heavy traffic road in Muscat, Oman, on two aquatic organisms (*Daphnia magna* & *Artemia salina*) and two native plant species (*Prosopis cineraria* & *Vachellia tortilis*).

2. Methodology

2.1. Dust Samples Collection and Analysis

Road dust samples have been collected from heavy traffic road (Sultan Qaboos Road) from Muscat the capital of Oman. Dust samples were collected in triplicate using cordless vacuum cleaners and then stored in pre-labeled, self-sealing polyethylene bags. Runoff samples were collected from the same road during rain as a first flush for analysis of heavy metals and nutrients. ICP-OES was used to determine the concentration of trace metals in the dust extract, the artificial mixture and the runoff sample. Sample digestion and analysis are carried out according to the standard methods [23]. Dust extract was analyzed using IC (Ion Chromatography) to detect the concentration of nitrate and potassium as source of nutrients for root germination. To confirm the nutrients results in the extracted dust, road runoff sample was analyzed using IC to determine the content of nitrate and potassium. Artificial mixture was made of standard fresh/ or sea water and six trace metals (Hg, As, Pb, Cu, Cr and Zn) according to the EC50 of each metal reported in the study conducted by [24].

2.2. Daphnia

Daphnia magna is usually used as a model organism for studying environmental exposure due to its vulnerability to environmental contaminants and ease to maintain in laboratory cultures [25]. *Daphnia magna* cultures have been initiated in the SQU (Sultan Qaboos University) Lab, biology department. The cultures have been maintained according to the standard condition during experimental period. *Daphnia magna* lives in freshwater therefore, standard freshwater with similar physicochemical parameters were prepared. Four solutions were prepared according to the international organization for standardization (ISO 2012). Initially, one-Liter solutions were separately prepared for calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), sodium bicarbonate ($NaHCO_3$) and potassium chloride (KCl) by dissolving 11.76, 4.93, 2.59, and 0.23 g of each salt respectively in distilled water. Then, 25 mL from each solution is added to volumetric flask and diluted with distilled water to a total volume of 1000 mL. Later, the diluted water was aerated using diluted pumps. Water is then transferred to food-grade containers each one 500 mL plastic container where *D. magna* are maintained. These minerals and/or ions are essential for the growth and development of *D. magna*. Ten Larval daphnids (neonates less than 24-hours old) were placed in individual containers (wells) and exposed to dust extracts and artificial mixture over a 48-hours period. Ten grams of road dust was added into a total volume of 50 mL of standard fresh water and kept for 1 hour and/or 24 hours as holding time before exposure. According to [8], the holding time is the time of storing the mixture of dust water extract for certain time at 25˚C in dark prior to the toxicity test. The different holding time (1 and 24 h) were taken to estimate the leaching characteristics and bioavailability of the metals after different holding time. During holding time of toxicity test, there is a likelihood of formation of ions that might enhance toxic effect. Each well was filled with 10 mL of the respective concentration of road dust extract and/ or artificial mixture. Mortality was the endpoint of the test. A series of assays were tried to evaluate the response to the toxicity of road dust in Daphnia magna (Figure 1). This includes 12 tests with alteration in various factors such as holding time of the extract/mixture, extract filtration, the artificial mixture of selected heavy metals, fixed pH, and direct contact test. Filtration of road dust extract has been carried out using nylon 66 membranes 0.45 µm × 47 mm. To simulate the rain pH (normal rain 5.6) extraction has been conducted using standard fresh water in pH 6.

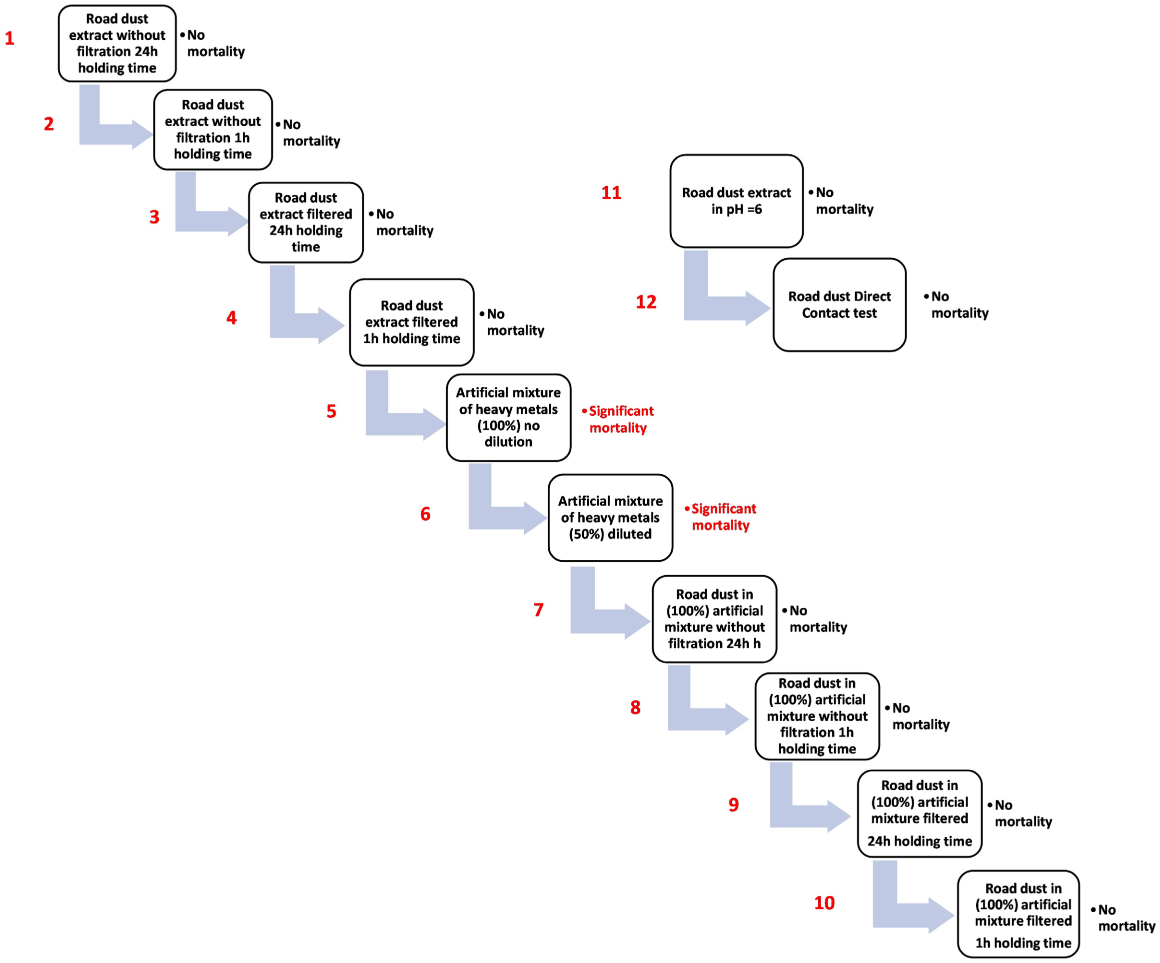

Figure 1. Different scenarios of the toxicity experiments conducted on *Daphnia magna* and *Artemia salina*.

2.3. Brine Shrimp

Artemia salina (brine shrimp) lives in saline waters and has been considered as standard test organism laterally with *Daphnia* species and widely used to evaluate the acute toxicity of different pollutants [24] [25]. The cysts of *Artemia salina* were hatched under standard conditions. *A. salina* was cultured by adding 0.3 g of solid eggs in 2 L distilled water mixed with 25 g of sea salt. Then, this mixture was kept under light and aeration for three days. Newly hatched nauplii were transferred into clean beakers. The larvae were used for toxicity assay at 24 h post-hatch. Artificial sea water was prepared as stock solution used for dust extraction and 10 individual *A. salina* nauplii were transferred with a pipette and placed in each well in the container test. The initial test was carried out with 3 replicates. An additional container with 10 nauplii in 10 mL artificial seawater was included as control. Ten nauplii of brine shrimp were placed into each well container. The container was placed in controlled temperature of 25 °C for 48 h. Toxicity of the road dust was examined on *Artemia salina* using the same tests mentioned in **Figure 1** except the test number 5, 6 and 12, respectively. Mortality was the endpoint of the test.

2.4. Seed Germination

Seeds of *Prosopis cineraria* and *Vachellia tortilis* were collected and stored in dry and dark conditions in the lab. Seeds were disinfected before treatments by soaking in a solution of 1 % sodium hypochlorite for two minutes and completely washed (3 times) with sterile water before the germination treatment to avoid any type of fungal contamination during germination. Ten seeds were randomly selected and placed in Petri dish (90 mm diameter) contained filter paper (Whatman No. 42) moistened with 5 mL of distilled water as a control and 5 mL of road dust extraction (different amount of dust in total volume of 50 mL distil water for 24 h). Three different concentration of road dust extract have been examined (2%, 20 % and 50 %), respectively. Experiments were conducted in triplicates and the number of seeds germinated was noted during 10 days until the maximum germination of the control group (distilled water). Seeds were considered as germinated on the first appearance of the radicle. Three parameters were calculated including Germination Percentage (GP) = (Number of germinated seeds/ Total number of seeds), Seedling Length (cm) and Seedling vigour index (SVI) (Seedling length (cm) x Germination percentage).

3. Results and Discussion

3.1. Toxicity on Aquatic Organisms

Survival of *Daphnia magna* and *Artemia salina* in non-filtered and filtered road dust extract in two different holding times are shown in **Figure 2**. It can be observed that there was no difference in the survival percentage between the control and different extract exposure. Even though the concentration of some metals such as Cu in the dust extract (0.07) exceeded the EC50 dose (0.02) mg/L, juveniles of both species did not show any mortalities during the experiments (**Table 1**). **Figure 3** shows the survival of *Daphnia magna* and *Artemia salina* in 100% (A) and *Daphnia magna* in 50% (B) artificial mixture of heavy metals. A significant decrease in the juveniles of *Daphnia magna* occurred after 24 and 48 hours in 100 % and 50% artificial mixture, respectively. On the other hand, 100% artificial mixture did not cause any mortalities on the nauplii of *Artemia salina* and that may be due to the concentration of metals being lower than the EC50 dose (**Table**

2). Furthermore, brine shrimp (*Artemia salina*) seems to be more tolerant to the artificial mixture of trace metals. With respect to different aquatic crustaceans, *Artemia* is highly resistant toward a variety of pollutants [26] [27]. For instance, toxicity of cadmium for *Daphnia magna* was four times higher than *A. fransiscana* [28]. The survival of *Daphnia magna* and *Artemia salina* in non-filtered and filtered road dust extracted by 100 % artificial mixture of heavy metals are illustrated in **Figure 4 (A)(B)**.

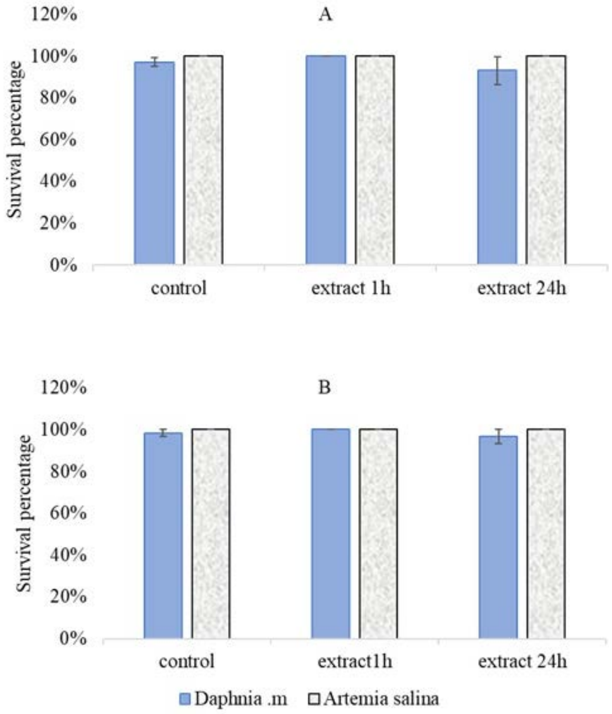

Figure 2. survival of *Daphnia magna* and *Artemia salina* in non-filtered (A) and filtered (B) dust extract in two different holding time 1 and 24 h.

Table 1. Chemical analysis of dust extract and mixture in different scenarios of *Daphnia magna* experiment (mg/L).

	Cr	Cu	Pb	Zn	As	Hg	Cr
Control	ND	ND	ND	ND	ND	ND	ND
Dust extract 1 h/filtered	ND	0.078	ND	0.133	ND	ND	ND
Dust extract 24 h/filtered	0.032	0.066	ND	0.143	ND	ND	0.032
Dust extract 1 h/nonfiltered	ND	0.071	ND	0.129	0.008	ND	ND
Dust extract 24 h/nonfiltered	0.039	0.071	ND	0.131	0.01	ND	0.039
100% Mixture	ND	0.02	ND	ND	1.959	ND	ND
50% Mixture	ND	0.009	ND	0.055	1.115	ND	ND
Mixture & dust 1 h/nonfiltered	0.067	0.066	ND	0.155	1.101	ND	0.067
Mixture & dust 24 h/nonfiltered	0.065	0.067	ND	0.117	1.106	ND	0.065
Mixture & dust 1 h/filtered	ND	0.057	ND	0.093	1.763	ND	ND
Mixture & dust 24 h/filtered	ND	0.043	ND	0.122	1.634	ND	ND
pH = 6	0.003	0.084	ND	0.06	0.009	ND	0.003
Direct contact	ND	0.121	ND	0.157	0.028	ND	ND

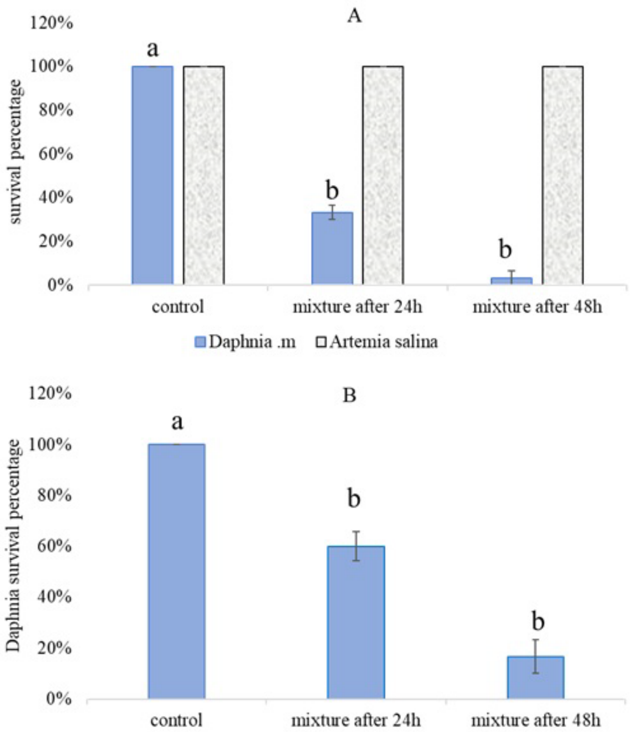

Figure 3. survival of *Daphnia magna* and *Artemia salina* in 100 % (A) and *Daphnia magna* in 50% (B) artificial mixture of heavy metals.

No significant change was reported in the survival percentage of both species during 48 hours of the experiments. In a study by [9] the survival of *D. magna* exposed to both filtered and unfiltered exposure was statistically similar. The exposure of dust extracted by the artificial mixture had no impact on the survival of organisms compared with exposure of 100% artificial mixture. **Table 1** shows considerable values of trace metals in the dust/mixture extracts (filtered and non-filtered). However, this exposure of the dust/mixture did not cause any mortalities in either species.

Figure 5 illustrates the survival of *Daphnia magna* in two different scenarios, road dust extract of pH = 6 and direct contact test. Although the concentration of trace metals in the two extracts was considerable, there was no change in the survival percentage between the control and the two exposures (**Table 2**). Dissolved Cu significantly contributed to mortality of *D. magna* however, particulate Cu, particulate Zn, and dissolved Zn did not show significant mortalities [9]. According to the aforementioned results we may conclude that the trace metals in the dust extract were probably not bioavailable, and these metals cannot be captured by the receptors [29].

On the other hand, the fractions of the concentrations of trace metals in the artificial mixture were possibly bioavailable and absorbed by the body of juveniles which caused high mortality percentage (**Figure 3**). However, 100% dust/mixture did not show any mortalities in the organisms. This might be due to the fact that the dissolved metal ions may adhere to the sediment via adsorption and ion exchange, thereby making the metal less bioavailable [30].

Table 2. Chemical analysis of dust extract and mixture in different scenarios of *Artemia salina* experiments (mg/L).

	Cr	Cu	Pb	Zn	As	Hg
Control	ND	ND	ND	ND	ND	ND
Dust extract 1 h /filtered	ND	0.372	ND	0.121	0.023	ND
Dust extract 24 h /filtered	0.01	0.242	ND	0.136	0.011	ND
Dust extract 1 h /nonfiltered	ND	0.234	ND	0.131	0.028	ND
Dust extract 24 h /nonfiltered	ND	0.218	ND	0.105	0.029	ND
100% mixture	ND	0.062	ND	0.064	1.766	ND
Mixture & dust 1 h/nonfiltered	ND	0.094	ND	0.097	1.429	ND
Mixture & dust 24 h/nonfiltered	0.007	0.15	ND	0.109	0.945	ND
Mixture & dust 1 h/filtered	ND	0.124	ND	0.077	1.437	ND
Mixture & dust 24 h/filtered	0.008	0.119	ND	0.113	1.163	ND

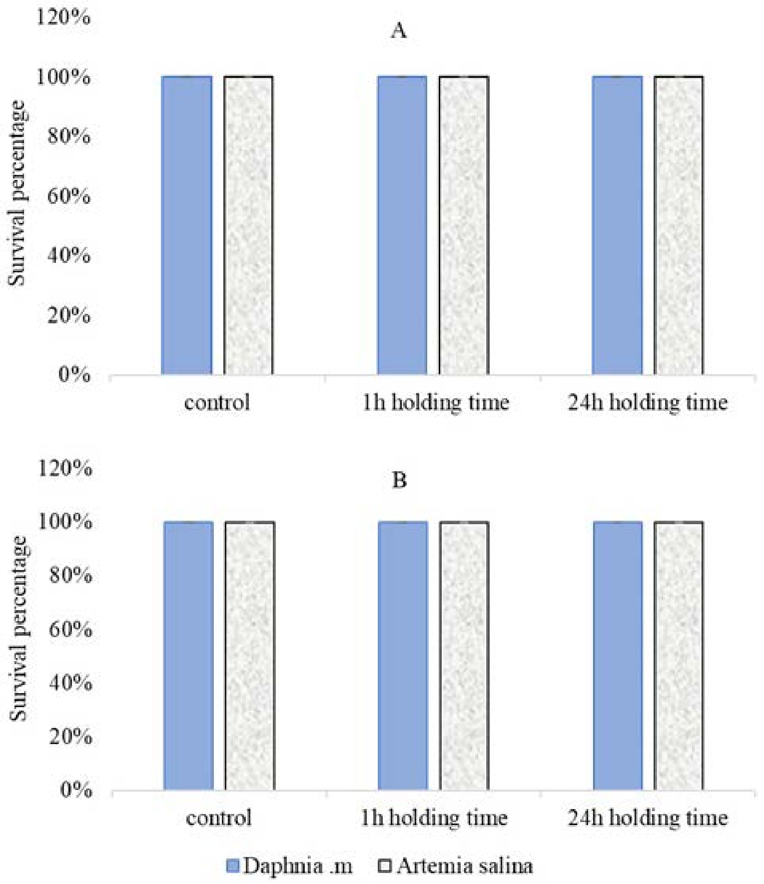

Figure 4. Survival of *Daphnia magna* and *Artemia salina* in non-filtered (A) and filtered (B) road dust extracted by 100% artificial mixture of heavy metals.

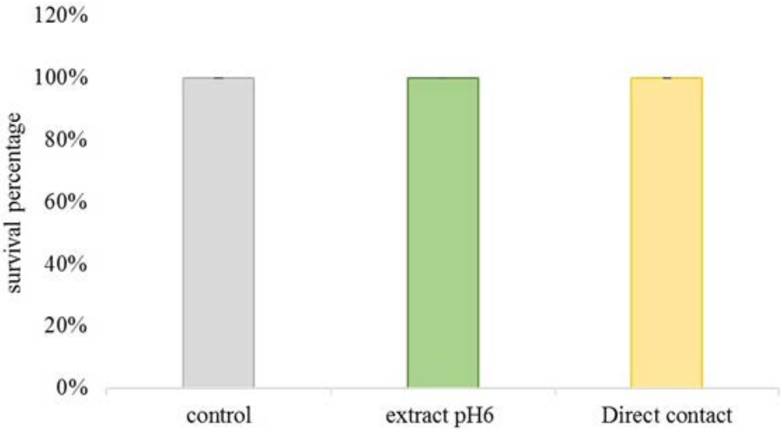

Figure 5. Survival of *Daphnia magna* in pH = 6 and in direct contact with the road dust.

3.2. Toxicity on Seed Germination

Figure 6 illustrates the germination percentage of *Prosopis cineraria* and *Vachellia tortilis* in three different road dust extract concentrations. Significant difference was obtained in the germination percentage between the control and three different extract exposures in both plant species. While no significant differences in the germination percentages among the three extract exposures in each species. Comparing two plant species, *P. cineraria* germination seeds was inhibited more than *V. tortilis* and the germination percentage of *P. cineraria* in 50% extract was significantly lower than *V. tortilis*.

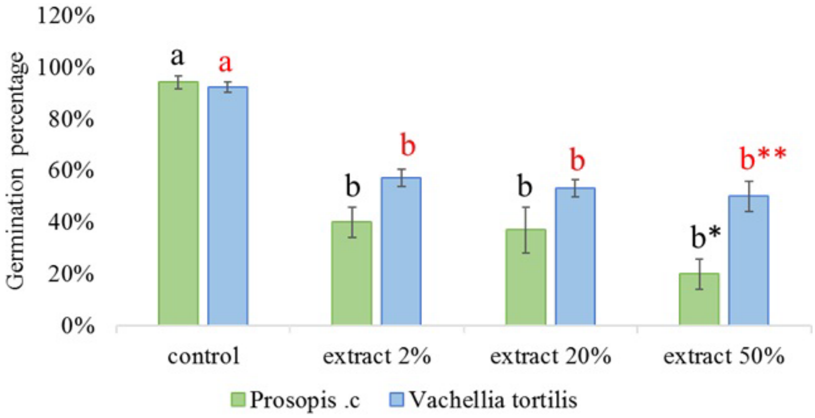

Figure 6. Germination percentage of *Prosopis cineraria* and *Vachellia tortilis* in three different road dust extract concentrations.

In a study by [21] reported that *Acacia* species have the ability to tolerate and accumulate heavy metals in a different part of the plant. Analysis of the different extract exposures shows varied concentrations of trace metals with the highest amount recorded for Hg in the 20% extract followed by Cu in the extract of 50% (**Table 3**). Although seeds show some tolerance in the presence of different trace metals, the percentage of germinated seeds depends on the metal content and the kind of plant species [31].

Table 3. Chemical analysis of road runoff and dust extract in three different concentrations (mg/L).

	Cr	Cu	Pb	Zn	As	Hg	Potassium	Nitrate	Phosphate
Control	ND	ND	ND	ND	ND	ND	ND	ND	ND
Extract 2 %	ND	0.036	ND	0.159	0.01	0.15	1.574	3.005	ND
Extract 20 %	0.012	0.084	ND	0.153	0.002	0.258	15.802	15.079	ND
Extract 50 %	0.048	0.179	ND	0.131	0.021	0.12	15.615	8.039	ND
Runoff	0.005	0.106	ND	0.087	0.002	ND	1.383	50.804	ND

Figure 7 shows the mean root length of *Prosopis cineraria* and *Vachellia tortilis* in three different road dust extract concentrations. No significant difference in the mean root length was observed between the control and the three different extract exposures in the two species (**Figure 7**). The mean root length of *P. cineraria* and *V. totilis* in the extract of 20% and 50% was higher than the mean root length of the control. The root system is responsive to nutrient availability and distribution within the soil [32]. Root elongation in the extract of 20% and 50% was significantly stimulated via potassium and nitrate presented in the exposures (**Table 3**). [33] suggested that the improvement of potassium nutritional status of plants might have a significant role for the survival of plants under different stress conditions.

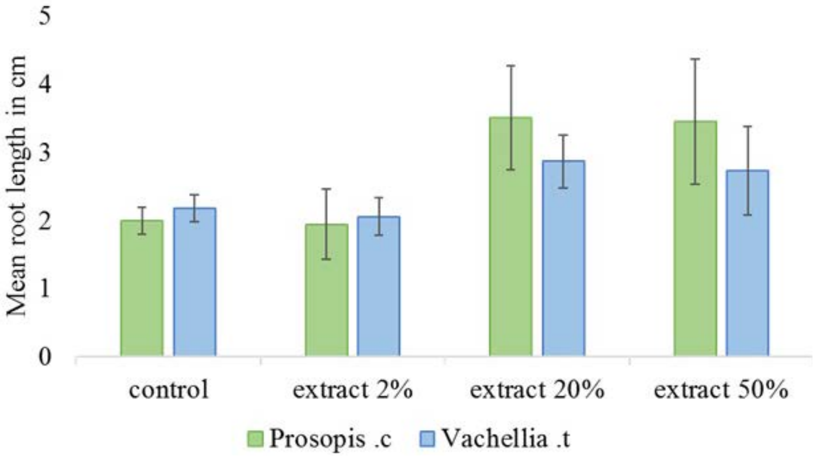

Figure 7. Root length of *Prosopis cineraria* and *Vachellia tortilis* in three different road dust extract concentrations.

Seedling vigor index of *Prosopis cineraria* and *Vachellia tortilis* in different extract concentrations is illustrated in **Figure 8**. Seedling vigor index (SVI) is a measure of the level of damage that accumulates as viability drops, and the seeds are incapable of germinating and finally die [34]. Interestingly, in contrast to the results of root length, seedling vigor indices of both species in the control were significantly higher than that of *P. cineraria* and *V. tortilis* in the extract of 2% (**Figure 8**). The seedling vigor index was higher in the control and lower in the extracts that contained heavy metals. In the study conducted by [35], seedling vigor index has increased at lower metal concentration and decreased when the concentration of metals was higher. Studies show that the seedling vigor index has been widely used as a phytotoxicity index to estimate the heavy metal impact on the seedling growth [36] [37].

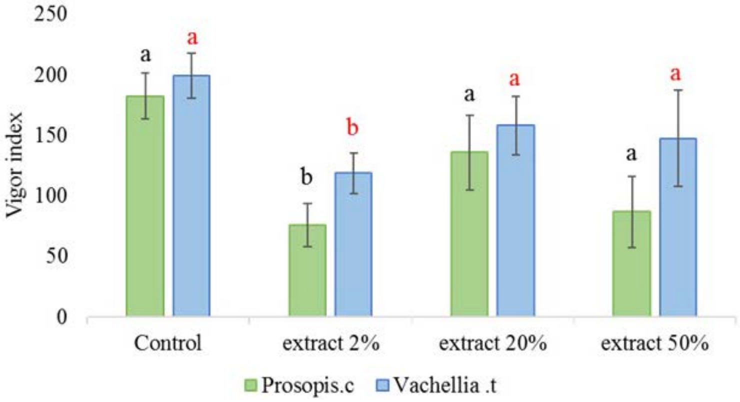

Figure 8. Seedling vigor index of *Prosopis cineraria* and *Vachellia tortilis* in different extract concentrations.

4. Conclusion

Direct exposure of *D. magna* and *A. salina* to road dust revealed that there was no significant impact on the survival percentage between the control and all scenarios until the artificial mixture was introduced that caused significant mortality in *D. magna* juveniles. Although the concentration of trace metals in the different extract exposures was considerable, no significant mortalities were observed in the dust extract exposures. Thus, the trace metals in the dust extract associated with suspended sediment were most likely not bioavailable and the metals were not absorbed by the receptors. However, the fractions of concentration of trace metals in the artificial mixture might be readily bioavailable and absorbed by the body of juveniles which caused a high percentage of mortality. On the other hand, in the plant toxicity test, significant difference was obtained in the germination percentage between the control and three different extract exposures in both plant species. Germination percentage of *P. cineraria* in 50% extract was significantly lower than *V. tortilis* showing the impact of the dust particles on *P. cineraria.* However, Seedling vigor index was lower in the extract containing heavy metals compared to the control showing the effects of heavy metals on the quality of seed vigor in general. This type of toxicity studies using directly the source of contamination is rarely attempted to see the impact of atmospheric particles. Though it is challenging to set the experimental design until some observable response, it is still worth trying logical stepwise scenarios either by varying the amount of dust or concentration of trace metals in the dust particles, leading to potential insight on the plausible impacts of atmospheric particles on the receiving environment.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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