Experimental Hydrogen Sulphide Deposition on Dominant Plants around Geothermal Power Plants in Kenya and Iceland

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Keywords: Hydrogen sulphide emissions, geothermal power plants, Sulphur accumulation, plant growth, *Tarchonanthus camphoratus, Racomitrium lanuginosum*

ABSTRACT

Previous studies show that plants close to geothermal power plants can accumulate sulphur from emitted H_2S gas, but the responses in terms of growth and physiology are not well described. We carried out two separate controlled experiments on dominant plants around geothermal power plants in Kenya and Iceland, i.e. the shrub *Tarchonathus camphoratus* (using seedlings) and the moss *Racomitrium lanuginosum*, respectively. We tested the hypothesis that sulphur concentration and accumulation in plant tissues would increase with increasing concentrations of wet hydrogen sulphide deposition, with consequences for plant growth and health. We irrigated the plants with 0, 30, 100 and 300 µg /L hydrogen sulphide gas dissolved in distilled water, for 6.5 (shrub) and 13 (moss) weeks, and measured plant responses in terms of sulphur concentrations (and calculated accumulation), foliar damage, growth, chlorophyll concentrations and contents (total amount). Due to lack of roots and their sensitivity to atmospheric depositions, we expected mosses to respond more strongly than the shrub. The treatments did not affect sulphur concentrations and accumulation in shrub leaves, nor did they affect foliar damage or chlorophyll concentrations and content of seedlings. However, stem height increase was greatest at intermediate H_2S exposure. The treatments had no effect on sulphur concentration and accumulation, biomass increase or chlorophyll concentrations/contents of moss shoots, but shoot length was reduced at high H_2S concentration exposures. We thus conclude that short-term exposure to moderate levels of H_2S (watering with 30 µg/L H_2S solution) is not harmful to either of the two plants and may even stimulate shrub growth while high levels may reduce moss growth.

1. INTRODUCTION

Hydrogen sulphide (H_2S) gas from geothermal power plants using hot water and steam for electricity generation, is the second most abundant non-condensable gas (NCG) emitted after carbon dioxide. Generally, it constitutes 1- 5% w/w of the NCGs fraction but may in rare cases reach up to 24% (Axtmann, 1975; Rodríguez, 2014). Its deposition (in various forms of sulphur) and sulphur accumulation in terrestrial ecosystems around geothermal power plants is a growing concern (Bargagli et al., 1997; Bussotti et al., 2003, 1997; Mutia et al., 2016a, b), particularly because of its potential phyto-toxicity at elevated levels (Bussotti et al., 1997).

Emissions of H_2S from geothermal power plants may have severe ecological consequences as wet or gaseous deposition (Kellogg et al. 1972). H_2S gas is relatively unstable in air and can undergo oxidation to form SO_2, and with even further reactions to produce sulphuric acid (Kellogg et al., 1972). In the presence of precipitation, the likelihood of acid precipitation around the power plants or further away is high but depends on the wind dispersion of the gas. This is of concern as acid precipitation can affect plant growth, survival and nutrient cycling in ecosystems (Likens et al., 1972).

Plants and lichens have been used as bio-monitors to map the deposition and distributions of geothermally emitted elements from geothermal power plants (Bargagli et al., 1997; Bussotti et al., 2003, 1997; Mutia et al., 2016a, b). These studies have shown clear patterns of increased sulphur concentration in plants and lichens close to the power plants. However, the associated effects of increased H_2S levels on the growth and physiology of plants remain unclear. Some indications of leaf damage including necrosis, chlorosis, reduced growth and early senescence have been reported (Varshney et al., 1979; Bussotti et al., 1997; Chiarucci et al., 2008; Bussotti et al., 2003; Mutia et al., 2016a, b), but still it is not established whether these are caused by excess sulphur or other environmental factors (Bussotti et al., 1997).

Assessment of the effects of a single pollutant on plant growth in the field is complicated by several interacting biotic and abiotic factors, including other pollutants. Only a few experimental studies have evaluated the effects of H_2S gas emissions on plants, mostly using vascular plants in fumigation experiments (Thompson and Kats, 1978; Gonzales, 1984; Maas et al., 1987, 1988). For example, Thompson and Kats, (1978) assessed the effects of continuous fumigation of H_2S on crops and forest plants in a greenhouse, and showed stimulated plant growth at 30 – 100 ppb H_2S gas and damaging effects at 300 – 3000 ppb. The damages were characterized by leaf lesions, defoliation and stunted growth (Thompson and Kats, 1978). It is worth noting that at optimum concentrations (variable between plants), sulphur serves as a macro-nutrient essential for plant metabolism and growth, but becomes phyto-toxic at high levels (Linzon et al., 1979). Other studies report decreased plant growth with H_2S fumigation at different concentrations, although the effects vary across different species (Gonzales, 1984; Maas et al., 1987). On the other hand, experimental studies on the effects of H_2S gas on non-vascular plants, such as mosses, which dominate plant communities in many subarctic ecosystems, are limited. Existing studies are mainly based on field surveys that assess sulphur levels in mosses growing around geothermal power plants (Baldi, 1988; Berg and Steinnes, 1997; Bargagli et al., 2002; Mutia et al., 2016b). There is more information, however, on sulphur effects related to experimental sulphur dioxide (SO_2) deposition on mosses. A study by LeBlanc and Rao, (1973) revealed damages on the mosses

Orthotrichum obtusifolium and *Pylaisia polyantha* at 5 and 30 ppb SO_2 exposures: moss colour changed from green to golden brown and leaf cells were plasmolysed.

In the design and implementation of appropriate mitigation measures toward sustainable geothermal development in Kenya and Iceland, where there are on-going and future geothermal development plans (Omenda et al., 2014; Ragnarsson, 2015), it is important to establish the effects of the power plant emissions. The aim of this study is therefore to assess plant responses to excess sulphur (in H_2S form) in dominant plants around geothermal power plants in Kenya (Mutia et al., 2016a) and Iceland (Mutia et al., 2016b) by experimentally evaluating causal relationships between wet H_2S and plant growth and health. Since existing experimental studies have assessed the effect of dry H_2S deposition (fumigation experiments) on plants, we chose to study effects related to wet H_2S deposition. This is owing to limited knowledge on this, and the fact that around power plants emitted H_2S may dissolve in precipitation before deposition in ecosystems.

We studied the effect of wet hydrogen sulphide deposition at different concentrations on two plant species: seedlings of the shrub *Tarchonanthus camphoratus* L., dominant around geothermal power plants in Kenya, and the moss *Racomitrium lanuginosum* (Hedw.) Brid., dominant around the Hengill geothermal power plants in Iceland. We hypothesized that with increasing concentration of wet H_2S exposure, sulphur would be enriched and accumulate in the plants with consequences for plant growth and health. Specifically, we expected sulphur enrichment in plant tissue when exposed to high H_2S concentration (> 30 μg/L) leading to increased foliar damage, reduced plant growth and decreased chlorophyll levels. We expected stronger responses in *R. lanuginosum* than in the *T. camphoratus* seedlings due to lack of roots and general sensitivity of mosses to atmospheric pollutants (Rao, 1982).

2. MATERIALS AND METHODS

We carried out two experiments, one in each country. In Kenya, the experiment was performed on seedlings of the shrub *T. camphoratus* (Experiment 1) and in Iceland on extracted moss mats of *R. lanuginosum* (Experiment 2).

We prepared H_2S treatment solutions at 0, 30, 100 and 300 μg/L (ppb) concentrations in distilled water. These solutions correspond to air saturated water with H_2S concentrations in air of 0 ppm, 10.96 ppm, 36.52 ppm, and 109.57 ppm, respectively (using a Henry's law constant of 0.001 mol/(L*atm), (Sander, 2015)). To prepare the treatments, a H_2S stock solution was initially prepared from 5% hydrogen sulphide gas in nitrogen. The gas was first bubbled in a 20 L distilled water container for 20 minutes and pH measurements taken at 5 minute intervals until there was no change (saturation). H_2S concentration in the stock solution was then determined using mercury acetate, according to methods described in Arnórsson et al. (2006), and ranged between 3.0×10^4 and 4.0×10^4 μg/L. From the stock solution, we prepared the H_2S gas concentrations for the treatments and further confirmed their concentrations via titration (Arnórsson et al., 2006). The solutions were made immediately before each use.

For each experiment, a similar nested design was adopted with four units randomly assigned to each treatment and multiple measurements conducted per unit.

2.1 Experiment 1: set-up and design

Seeds of *T. camphoratus* were obtained from the Menengai forest in Kenya (0.2500° S, 36.0833° E, 2278 m a.s.l.). The forest grows and extends over the Menengai caldera, a trachytic volcano in the Kenya Rift Valley (Leat, 1984). The area presently has no geothermal power plants and is under exploration for geothermal development, with drilling of geothermal wells ongoing. Seeds were sown at a tree nursery within the caldera between August and September 2014 and the seedlings pricked after one month, and potted in perforated polythene bags (13 cm x 20 cm) filled with volcanic soil of sandy texture obtained from the area, and nurtured at the tree nursery for a period of seven months, to attain optimum growth (3 – 4 true leaves) for transplanting and acclimation to environmental conditions.

Potted seedlings were later transferred to an outdoor open ground in Nakuru, Kenya (0.2777° S and 36.0504° E, 1889 m a.s.l) where the experiment was conducted between 18th March and 1st May 2014. Over the course of the experiment, seedlings were exposed to three rain events totaling 88 mm. The daily temperature ranges were between 17 and 20° C as measured at the Mlima Punda automatic weather station at Menengai (Geothermal Development Company Ltd, unpublished data 2014).

Seedlings were arranged in groups of ten per experimental unit, for a total of sixteen units. Within each unit, five seedlings were randomly chosen and labelled for measurements and sampling, and the remaining five served as reserves in case of mortality, for a total of 80 seedlings across all treatments.

The H_2S solutions were applied to the *T. camphoratus* seedlings. Ten liters of the solutions were applied per group four times a week using watering cans (simulating a rainfall event). The experiment was performed for a period of 6.5 weeks (45 days). The duration was chosen based on the growth rates of seedlings (average 3.92 cm stem height increase during the experiment), and that was assumed long enough to detect effects of the treatments.

2.1.1 Experiment 1: growth measurements, chlorophyll and sulphur determination

Growth related variables i.e. stem height, number of stems and number of healthy green leaves per seedling were measured/counted at the beginning and end of the experiment. Foliar damage was assessed at the end of the experiment based on leaf colour and appearance, as previously described in Mutia et al. (2016a): A) healthy green leaves, B) yellow leaves and C) brown dead leaves. The proportion of damaged leaves on each seedling was calculated as the number of leaves in categories B and C over the total number of leaves. Some seedlings had damaged leaves at the beginning of the experiment, but there were no differences in the proportion of damage among treatment groups prior to the experimental manipulations (Chisq=5.168, p=0.160), so we assumed that any potential differences at the end of the experiment would be due to the H_2S exposure. Foliar damage, i.e. the proportion of damaged leaves (categories B and C) in control seedlings at the beginning and the end of the experiment, was compared using a paired t-test

to assess the effect of time, independent of the experimental manipulations; results showed that the proportion of foliar damage did not significantly change over the course of the experiment (t = -2.011, p = 0.058). Although marginally significant, this may indicate that foliar damage occurred to the plants during the course of the experiment even though no treatment was applied.

At the end of the experiment, wearing polythene gloves, all leaves were carefully removed from each seedling and grouped according to the three damage categories. At the Geothermal Development Company Ltd (GDC) laboratory, each sample was washed in distilled water, dried at room temperature in the dark and divided into two parts, one for chlorophyll determination and the other for total sulphur analysis. Chlorophyll concentrations were determined in A and B leaves (not in the dead C leaves). For chlorophyll determination, each sample was milled, weighed and split into two sub samples, one for chlorophyll concentration analysis and the other for dry weight determination (after oven drying to a constant weight at 70 °C for 24 hours). Ten ml of 96% ethanol was added to 0.5 g of each sample and the mixture hand shaken for 15 seconds. The samples were then covered by aluminum foil to prevent light exposure and allowed to stand for 24 hours at 6 °C in darkness and centrifuged for 10 min at 1000 revolutions per min. 3.5 ml samples were extracted and transferred to 4 ml cuvettes for analysis at the Institute of Freshwater Fisheries in Iceland (modified from Sumanta et al., 2014). To determine chlorophyll concentrations, light absorbance at wavelengths of 750 nm, 663 nm and 652 nm was measured using a spectrophotometer (HACH LANGE UV Visible Spectrophotometer, DR 5000). Chlorophyll concentration in mg/g dry weight was calculated according to Arnon (1949). For sulphur determination, leaf samples were analysed using standard analytical procedures at the internationally accredited ALS Scandinavia labs in Luleå, Sweden. Prior to analysis, samples were acid digested (in 5 ml conc. HNO_3 + 0.5 ml 30% H_2O_2) in closed teflon containers in a microwave. Sulphur analyses were conducted using an Element 2 ICP MS according to (modified) U.S.EPA methods 200.8 (U.S.EPA, 1994) and SS EN ISO 17294 parts 1 (ISO, 2005) and 2 (ISO, 2003). Procedural blanks were below the minimum detection level. Accuracy was checked through analysis of standard in-house reference materials for soil (ALS Labs, Sweden) and peach leaves (NIST 1547) (National Institute of Standards and Technology, Gaithersburg, MD, USA; (Rodushkin et al., 2008) and obtained more than 95% recoveries. Estimates of sulphur accumulation and chlorophyll content (total amount) for each seedling were based on the number of leaves in each category, multiplied by the sulphur or chlorophyll concentration of that leaf category in that seedling, and the average leaf weight (0.015±0.002 grams, mean ± SE), and summed across all leaf categories. Concentrations of sulphur in healthy leaves (category A) for each seedling are also compared with the sulphur concentration in the healthy moss.

To account for other factors that influence plant growth and health, we measured soil sulphur concentrations and soil characteristics i.e. pH and moisture (% by weight) in the seedling pots, 80 in total. Each sample was split into three, one for the analysis of sulphur concentration and the other for analysis of soil pH and moisture (% by weight). The samples for sulphur analysis were dried at 50 °C for 48 h to a constant weight, sieved through a 2 mm sieve and analysed using the same protocols and equipment as for the leaves at the ALS Scandinavia labs in Luleå, Sweden. For soil pH, soil solution was extracted from 5 g (<2 mm) of 96 hours air-dried soil in 25 ml de-ionized water, by shaking it for two hours and allowing to settle for 8 hours before measuring pH (Blakemore et al., 1987). Soil moisture (%) by mass was obtained after oven drying 10 g of fresh soil at 105 °C for 24 hours to constant weight.

2.2 Experiment 2: set-up and design

Sixteen 16 x 24 cm mats of the moss *R. lanuginosum* trimmed to a depth 5 cm were extracted from Raudhalsahraun, a lava field with no geothermal activity, located within the Snaefellsness volcanic belt (Thordarson and Larsen, 2007) in West Iceland (22.2640° W 64.8483° N) at 331 m above sea level. Plastic trays (16 x 24 x 8 cm) were filled with 3 cm tephra at the base (obtained from the same area as the moss) for use as growth substrate over which the extracted moss mats were placed. The experiment was carried out in a growth chamber at the University of Iceland in the late summer-autumn period (2nd August 2013 – 30th October 2013). We maintained constant conditions within the growth chambers with 12 hours day light exposure (Photosynthetically Active Radiation (PAR) 250 $\mu molm^{-2}s^{-1}$) and air temperatures between 17 and 20 °C. Optimal growth temperatures for *R. lanuginosum* of 5–13 °C (Tallis, 1964; Kallio and Heinonen, 1973) could not be maintained in the chamber due to heat development from the photosynthetic light bulbs; for the same reason, we had to compromise the light period, even though *R. lanuginosum* grows under almost 24 light hours (Average PAR 170 $\mu molm^{-2}s^{-1}$) in the Icelandic summer.

The hydrogen sulphide treatment solutions were applied to *R. lanuginosum* moss mats. 300 ml of the solutions were applied in each tray four times a week using a mist sprayer, with four replicate trays per treatment. This experiment was conducted for a longer period (90 days, August – October 2013) than experiment 1, owing to the slow growth of mosses.

2.2.1 Experiment 2: shoot growth, moss damage assessments, chlorophyll, and sulphur determination

Moss growth was assessed as shoot length increase and biomass increase over the experimental period using open-ended netlon bags (Jónsdóttir et al. 1999, Armitage et al. 2012). For each bag, twenty fresh moss shoots of *R. lanuginosum* were collected from the same area as the moss mats (Raudhalsahraun) and trimmed to 30 mm apical length. Ten of these shoots were weighed fresh and placed in the tagged bag and carefully inserted into the moss mats within the trays at the beginning (t_0) of the experiment; one bag was included per tray. The other ten shoots were used to determine the ratio between fresh and dry weight (after drying to constant weight at 70 °C) to calculate the dry weight of the transplanted shoots at the beginning of the experiment (Jónsdóttir et al., 1999). The transplanted shoots were left in the moss mats until the end of the experiment (t_1). Shoot growth was measured as shoot length increase in excess of the original 30 mm, for each shoot. After the experiment, the same shoots were dried at 70 °C to obtain the dry weight at time t_1. Biomass increase was calculated collectively for the ten shoots per netlon bag, by subtracting the calculated dry weight of the ten shoots at time t_0 from the dry weight at time t_1. Moss foliar damage was assessed on a weekly basis by inspecting the colour and appearance of the moss shoots, as described in Mutia et al., (2016b): A) healthy green shoots, B) yellow shoots and C) brown/black dead shoots.

At the end of the experiment, three moss samples were systematically extracted from each tray, at the mid-point and both ends of the tray, and each sample divided into two parts/ subsamples, one for analysis of sulphur concentrations and the other for chlorophyll determination. For all samples, only the uppermost 3 cm (most photosynthetically active) of the moss shoots were used in the analysis. Further sample preparations and analysis in the shoots were performed in the same way and in the same labs as in experiment 1.

Calculations of sulphur accumulation and chlorophyll content for moss shoots were based on the biomass of the ten moss shoots in each tray at the end of experiment, multiplied by the average shoot concentrations of sulphur or chlorophyll per tray.

2.3 Data analyses

Sulphur concentration and accumulation in seedlings of *T. camphoratus*, foliar damage, growth measurements (stem height increase, change in number of stems, and change in number of healthy green leaves per seedling) and chlorophyll concentrations and content of seedlings were separately analysed using Linear Mixed effects Models (LMM) or Generalized Linear Mixed effects Models (GLMM). Count data (change in number of stems and number of green leaves) and proportional data (foliar damage) were analysed with GLMM using a poisson and binomial distribution, respectively. The experimental treatment (0, 30 µg/L, 100 µg/L and 300 µg/L) was included as a fixed factor and sampling units were included as a random factor. Soil characteristics (soil pH and% soil moisture) and soil sulphur concentrations were included as co-variates. The co-variates were included one at a time and then the best fitted model based on the lowest AIC (Akaike´s Information Criterion) value selected, provided that inclusion of an additional parameter in the model reduced the AIC value by more than 2.0. Significance of the variables in LMM and GLMM was calculated, comparing models with and without the variable of interest, so values are reported as F values and Chisq values, respectively.

Differences in sulphur and chlorophyll concentrations between leaf categories, and across the experimental treatments, were analysed with LMM, including sampling units as a random factor. As fixed factors we included the interaction between leaf category and experimental treatment. Co-variates were also included as indicated above. When the interaction was not significant, it was dropped from the final model.

Sulphur concentration and accumulation in moss shoots, growth measurements (shoot length increase and biomass increase), and chlorophyll concentration and content were analysed using Linear Models (LM) or LMM. LMs were used when one measurement was taken per sampling unit (sulphur accumulation, shoot biomass increase and chlorophyll concentration and content). The random factors included in the LMMs were 'tray' for sulphur concentration in *R. lanuginosum* shoots (3 measurements per tray) and sulphur accumulation and 'sampling bag' for shoot length increase measurements (one bag per tray, 10 shoots measured in each bag). Foliar damage of mosses was not analysed because moss colour change was only detected in one tray in the H_2S 30 µg/L treatment where the moss formed a brown colour patch (category C) after four weeks.

The models were run in R 3.2.2 (R Development Team, 2010) using the functions *lmer* in the lme4 package (Bates et al., 2014) for the LMM and *lm* in the MASS package (Ripley et al., 2015) for linear models. All plant variable and sulphur concentrations are summarised as mean ± standard error (SE).

3. RESULTS

3.1 Effect of wet H_2S treatments on *T. camphoratus* seedlings

On average, the S concentration in the leaves was 1639.44±94.38 mg/kg and the seedlings had accumulated 0.342±0.013 mg of sulphur in their leaves by the end of the experiment for all the treatments (average foliar dry mass per seedling was 0.233±0.050 grams). Sulphur concentration in the different leaf categories did not differ across the experimental treatments (Table 1, Figure 1a). Furthermore, the accumulation of sulphur in the seedlings was not significantly affected by the experimental exposure to increased wet H_2S depositions (Table 1, Figure 1b).

The increase in stem height was on average 3.92±0.14 cm for all treatments and was positively affected by the 30 µg/L treatment while it was not significantly different from the control at higher H_2S levels. (Figure 1c, Table 1).

By the end of the experiment the proportion of damaged leaves was on average 0.244 ± 0.013 for all treatments. Seedlings increased their number of stems by 1 ± 0.105 and on average, their number of green leaves decreased by one (-1 ± 0.477) for all treatments. Chlorophyll concentrations of the leaves averaged 1.45±0.09 mg g^{-1} for leaves A and 0.46±0.08 mg g^{-1} leaves B in all treatments. The average chlorophyll content (total amount) of seedlings was 0.240 ± 0.016 mg, ranging between 0.001 and 0.696 mg. Experimental exposure to wet H_2S deposition did not affect foliar damage (proportion of leaf damage), the number of stems and the number of green leaves (Table 1). Chlorophyll concentration was affected by the treatments, and as expected was at higher levels in the green healthy leaves than yellow leaves; leaves in the 30 µg/L and 100 µg/L treatments had lower chlorophyll levels than the control and the 300 µg/L treatment (Figure 1d). The total chlorophyll content of seedlings was, however, not affected by the experimental exposure (Table 1).

The soil co-variates did not differ across the experimental treatments (Table S1), but they improved some models by accounting for parts of un-explained variation (Table 1).

Table 1: Model results for the effects of H₂S exposure on responses of *T. camphoratus* seedlings: sulphur concentrations and accumulation, proportion of damaged leaves, stem height increase, change in number of new green leaves, change in number of stems and chlorophyll concentrations and contents. In all models, the effect of the experimental manipulation of H₂S exposure ('Treatment') was assessed; covariates ('Soil moisture', 'Soil sulphur' and 'Soil pH') were retained in the final model if they improved model fit. For LMMs, the numerator and denominator degrees of freedom are indicated. F values are reported for sulphur concentrations and accumulation and chlorophyll concentrations and contents per seedling (LMM) while Chisq values are given for the other response variables (GLMM).

| Response	Source of variation	Mean square	DF	F or Chisq value	p value
Sulphur concentrations in *T. camphoratus* leaves	Leaf category	1298366	2, 128	20.37	0.000
	Treatment	92570	3, 17	1.45	0.263
Sulphur accumulation in *T. camphoratus* per seedling	Treatment	0.01	3, 12	0.42	0.744
Stem height	Treatment	7.76	3, 17	4.12	**0.023**
	Soil sulphur	6.07	1, 163	3.22	0.075
	Soil pH	8.73	1, 166	4.64	**0.033**
Proportion of leaf damage	Treatment	1.98	3	5.17	0.584
Change in number of green leaves	Treatment	0.35	3	1.03	0.793
Change in number of stems	Treatment	1.13	3	3.15	0.370
	Soil sulphur	5.12	1	5.42	**0.020**
Chlorophyll concentrations in *T. camphoratus* leaves	Leaf category	17.9211	1, 106	34.57	0.000
	Treatment	1.5686	3, 106	3.03	**0.033**
	Soil moisture	2.5905	1, 106	5.00	**0.027**
Total chlorophyll content per seedling	Treatment	0.02	3	0.80	0.499
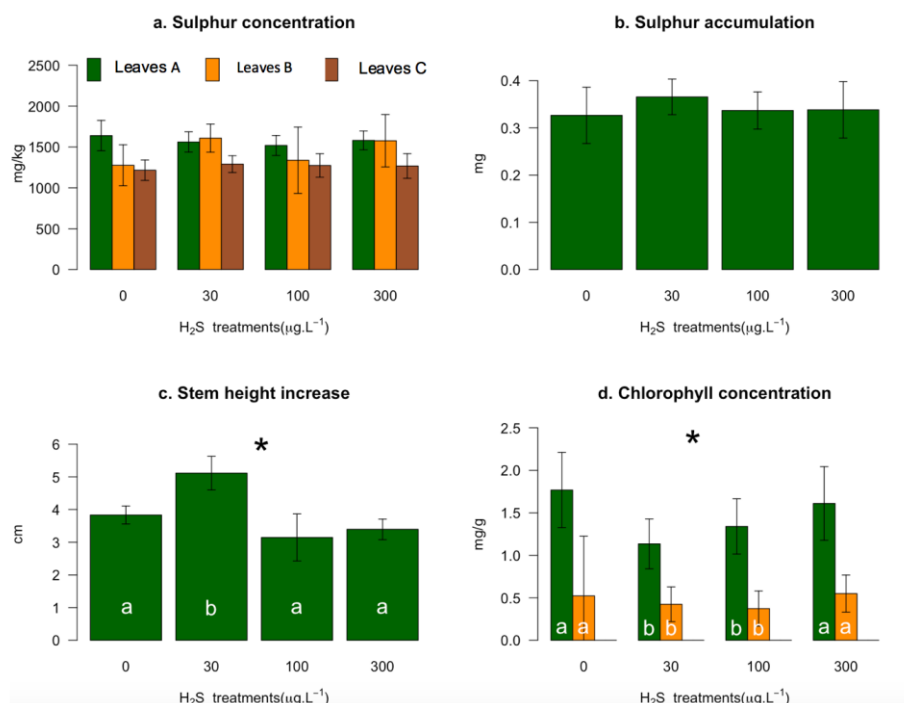
Figure 1. Various responses of *T. camphoratus* to H₂S treatments (application of 0 μg/L, 30 μg/L, 100 μg/L and 300 μg/L): (a) Sulphur concentrations in leaves, (b) sulphur accumulation per seedling, (c) stem height increase and (d) chlorophyll concentrations after 6.5 weeks in an outdoor experiment (mean ± SE, n = 4). Sulphur (a) and chlorophyll (d) concentrations in leaves of *Tarchonanthus camphoratus* are assigned to different damage categories based on visual assessment: healthy green leaves (leaves A), yellow leaves (leaves B) and dead brown leaves (leaves C), across the different H₂S experimental treatments. Asterisks (*) indicate significant effect of treatment (p<0.05) and smaller case letters show differences between treatments.

3.2 Effect of wet H₂S treatments to *R. lanuginosum* moss

The average sulphur concentration in moss was 205±4 mg/kg and the moss had accumulated 0.019±0.002 μg sulphur at the end of the experiment (average biomass weight per ten moss shoots was 0.09±0.006 mg) for all the treatments. The overall treatment effect on the concentration of sulphur in moss was marginally significant (Table 2), and was significantly lower at 30 μg/L wet H₂S deposition than at the higher exposures (Figure 2a), while the controls showed intermediate concentrations. The treatment did not affect sulphur accumulation in moss tissues (Table 2, Figure 2b).

Over the 90 days of the experiment, shoots elongated on average by 0.09±0.008 cm, biomass increased by 0.016±0.003 mg on average, and chlorophyll content of moss shoots ranged between 0.001 and 0.018 μg (average 0.008 ± 0.001 μg) for all treatments. Experimental exposure to H₂S had a significant effect on shoot length increase (Table 2; Figure 2c). Shoot length increase at the highest levels of exposure (300 μg/L H₂S) was significantly lower than at all other treatment levels. At these highest exposures, shoot length increase was reduced by 59%. The treatments did not affect moss biomass increase, or the chlorophyll concentrations and contents of moss shoots (Table 2, Figure 2d).

3.3 Comparison of sulphur concentration and accumulation between species

In general, mosses have low levels of nutrients (including sulphur) compared to vascular plant tissues, a reason why dead moss is recalcitrant. As such, the samples of *R. lanuginosum* showed much lower sulphur concentrations than seedlings of *T. camphoratus* (205 ± 4 mg/kg sulphur in moss vs 1441.407 ± 25.742 mg in the healthy leaves of *T. camphoratus* (leaves A)). However, contrary to our predictions the moss did neither become more enriched nor accumulate more sulphur in response to the treatments.

Table 2: Model results for the effects of H₂S exposure on responses of *R. lanuginosum*: sulphur concentrations and accumulation, biomass increase, shoot length increase and chlorophyll concentrations and contents. In all models, the effect of the experimental manipulation of H₂S exposure ('treatment') was assessed. For LMMs, numerator and denominator degrees of freedom are indicated.

| Response	Source of variation	Mean square	DF	F value	p value
Sulphur concentrations in R. lanuginosum	Treatment	1502.1	1,16	3.97	0.064
Sulphur accumulation in *R. lanuginosum*	Treatment	0.00	3	0.66	0.591
Biomass increase	Treatment	0.00	1	0.34	0.570
Shoot length increase	Treatment	0.04	3, 16	3.30	**0.048**
Chlorophyll concentrations in *R. lanuginosum*	Treatment	0.00	3.00	0.61	0.621
Total chlorophyll content per 10 shoots biomass weight	Treatment	0.00	3	0.36	0.782
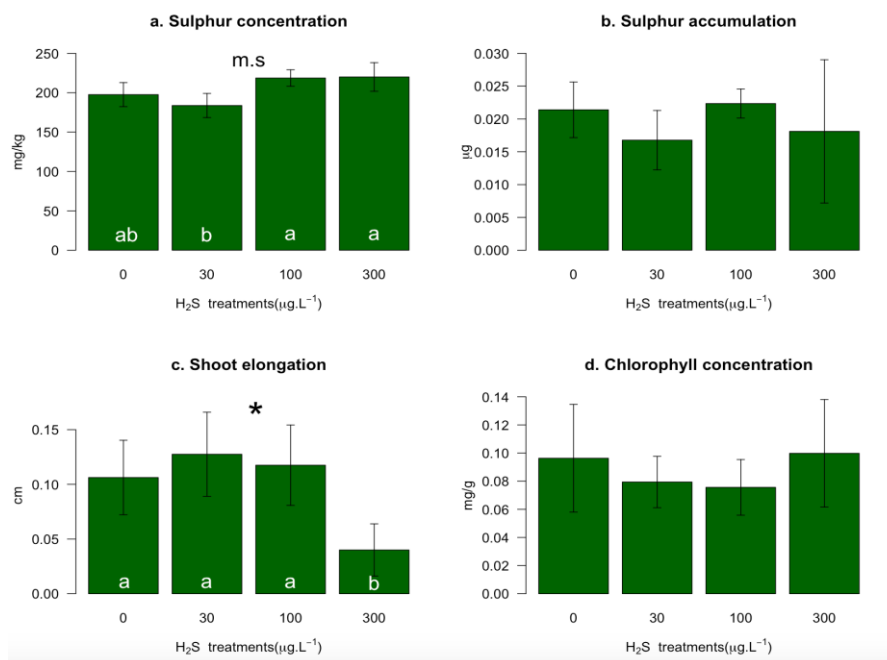
Figure 2. Various responses of *R. lanuginosum* to H₂S treatments (application of 0 μg/L, 30 μg/L, 100 μg/L and 300 μg/L): (a) Sulphur concentrations in shoots, (b) sulphur accumulation in shoots, (c) shoot elongation (shoot length increase) and (d) chlorophyll concentrations after 13 weeks in growth chambers (mean ± SE, n = 4). 'm.s' indicates a marginally significant effect of treatment (p=0.06), asterisks (*) indicate significant effect of treatment (p<0.05) and smaller case letters show differences between treatments.

4. DISCUSSION

Deposition and accumulation of sulphur in terrestrial ecosystems around geothermal power plants is a growing concern, because of the potential phyto-toxicity of sulphur at high levels. However, in the present study we found no clear evidence of accumulation of sulphur in response to experimentally increased wet H_2S deposition in the plants that dominate around geothermal plants, i.e. the shrub *T. camphoratus* in Kenya, and the moss *R. lanuginosum* in Iceland. However, the plants responded to the wet H_2S experimental exposure in terms of growth. According to our expectations, growth of *R. lanuginosum* decreased in response to high exposure levels. In the case of the shrub, there was an increase in stem height growth at intermediate concentrations of H_2S (30 µg/L).

Accumulation of sulphur in plants exposed to high concentrations of H_2S has been reported from field studies (Mutia et al., 2016a, b). In the present study, sulphur concentrations in the seedlings of *T. camphoratus* were about 70% higher than those measured in mature leaves of *T. camphoratus* in the field (Mutia et al., 2016a). This is not surprising; as mature leaves are poor sulphur sinks compared to young expanding leaves. Mature leaves preferentially redistribute sulphate to young expanding leaves (Rennenberg et al., 1979; Bell et al., 1995; Hartmann et al., 2000), roots (Sunarpi and Anderson, 1996) and generative sinks (seeds) for nutrition and growth. Such redistribution of sulphur was indeed indicated in our study by the reduced sulphur concentrations in the senescing and dead leaves. In contrast, *R. lanuginosum* showed sulphur values 35% higher in field samples (Mutia et al., 2016b) than in the present experiment. This may be partly explained by the duration of the experiment, with a shorter time exposure to H_2S compared to the continued exposure of mosses growing around power plants, and the environmental conditions during the experiment.

In general, there were no strong treatment effects on the responses of either plant, which was surprising, especially for the moss due to its susceptibility to air pollutants. Since mosses are slow growing, possibly a longer duration of the experiment would have shown more clearly the effects of excess sulphur in their tissues. For example, in a field experiment applying 1.0 mM (8.10 x 104 µg/L) bisulphite and 5.0 mM (4.8 x 105 µg/L) sulphate, marked sulphur accumulation and reduced shoot length increase in Sphagnum species were only evident after 18 months of treatment applications (Ferguson and Lee, 1980), so a period of over a year might be recommended in future studies. Still, even in the relatively short duration of our experiment (3 months) we already detected reduced shoot length increase at high H_2S exposures. Exposure to high concentration (100 µg/L and 300 µg/L) of H_2S treatment corresponded to increasing sulphur concentrations in *R. lanuginosum* (marginally significant) that matched slow shoot growth at high treatment exposure levels (300 µg/L). This yields some indication that the high sulphur concentrations may have had a negative influence on the shoot growth, in agreement with other studies (Ferguson et al., 1978; Ferguson and Lee, 1980).

In the case of *T. camphoratus*, the 30 µg/L treatment seemed to have a positive effect (fertilising effect), through stimulated shoot height. This is comparable to the findings of a H_2S fumigation experiment in Thompson and Kats, (1978), where 30 ppb significantly stimulated the growth of lettuce, sugar beets and alfalfa.

Overall, healthy leaves of *T. camphoratus* showed higher sulphur levels than the moss shoots in response to the treatments. This was opposite to what we anticipated, since mosses are more susceptible to atmospheric deposition (either as wet or dry deposition) of pollutants, so we expected the moss to accumulate more sulphur than the shrub. In the absence of pollution, this difference can be explained by the different plant mechanisms for nutrient absorption, where *T. camphoratus* acquires more nutrients (sulphur) from both the soil through roots and leaves than *R. lanuginosum*, which obtains all its nutrients (sulphur) from the air.

Other environmental factors, like the high light intensity and temperature conditions for *R. lanuginosum* during the experiment and the rain events for *T. camphoratus*, may help explain the weak responses we found. Strong light intensities cause photo-inhibition (Murata et al., 2007) and can destroy chlorophyll and DNA structures of bryophytes in moist condition (Glime, 2007). The concentrations of chlorophyll in our *R. lanuginosum* samples were similar to values measured in field (Mutia et al. 2016b), so we cannot unequivocally infer chlorophyll damage. However, high light intensity may have affected some other physiological processes within the mosses under these conditions. Similarly, the effect of temperatures higher than optimal (due to heating from the light bulbs) in the experimental growth chambers for *R. lanuginosum* could also have affected the moss responses to the treatment. To overcome these experimental limitations, we recommend that similar experiments are performed in better controlled environments or outdoors and away from geothermal activity or atmospheric pollution during their natural growth period, so that the moss grows in as close to natural conditions as possible. However, field experiments have other limitations. For example, in our outdoors experiment with shrub seedlings, the three rain events could have affected sulphur levels and other responses in the plant leaves through dilution and nutrient leaching. The measured soil variables, which improved the models, also suggest the influence of other environmental factors on the shrub growth and responses to the treatments.

5. CONCLUSION

We can therefore infer that short-term exposure to moderate levels of wet H_2S deposition (30 µg/L) does not harm the dominating plants around power plants in Kenya and Iceland. These levels of H_2S seemed to benefit growth in the case of the shrub, and did not reduce moss growth. However, caution needs to be taken with this interpretation and experiments assessing the long term effects of exposure should be conducted. In the case of R. lanuginosum, due to the high variability in the responses, experiments with larger numbers of replicates are required. Additional physiological plant responses such as photosynthetic rate (Maas et al., 1988) and changes in leaf area and dry matter for T. camphoratus (Maas et al., 1985; Bussotti et al., 2003) are advised. Fumigation experiments of the same H_2S concentrations are also encouraged on the plant species for comparison, especially to assess the threshold levels at which damages may be emergent.

proof reading of this manuscript. These investigations were supported in part by grants from the University of Iceland Research Fund and the UNU-GTP - Iceland.

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