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Elemental Sulfur Lowers Redox Potential and Produces Sulfide in Putting Green Sand

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Abstract. **Biological production of sulfide (S^{2-}) in soil has been reported to depend on system redox potential and on the concentrations of available sulfate (SO_4^{2-}) and organic carbon (OC). The purpose of this laboratory study was to determine whether elemental sulfur (S^o) could influence redox potential and S^{2-} production in sand used to construct putting greens. Treatment with S^o depressed redox potential as pe + pH, and stimulated accumulation of both free H_2S and acid-soluble S^{2-}. Organic carbon as lactate ($C_3H_5O_3Na$) intensified the effects of S^o, primarily by influencing pH. Thus, S^o application could induce anaerobiosis and subsequently affect turf quality by heightening production of free hydrogen sulfide (H_2S). It could also contribute to S^{2-} accumulation possibly expressed as a black layer or blackening of the root zone.**

A black banding or blackening of the root zone, termed black layer, has been observed in the soil profile of sand putting greens by turfgrass practitioners (Berndt et al., 1987; Gockel, 1987; Hodges, 1987a, 1987b, 1989; Lubin, 1987; Rankin, 1988; Scott, 1986; Smith, 1988). A decline in the quality of turfgrass has frequently been associated with the black layer (Berndt et al., 1987, 1989; Berndt and Vargas, 1989; Scott, 1986). We hypothesized that the black layer was an accumulation of precipitated sulfides (S^{2-}). We suggested that S^{2-} production could be stimulated by application of elemental sulfur (S^0) during periods of anaerobiosis (Berndt et al., 1987, 1989; Berndt and Vargas, 1989). Further, we postulated that S^o may contribute to anaerobiosis and that turf quality may decline due to occurrence of free hydrogen sulfide (H_2S), which is a known respiratory toxin (Atlas and Bartha, 1981). The objective of this research was to test the hypothesis that S^o could decrease redox potential and stimulate S^{2-} production in sands typically used to construct the root zone of golf putting greens.

For the experiment, 200 g sand was placed in 125-ml glass serum bottles. Sand particle size distribution was 99% of particles >0.1 mm in diameter and 84.5% of particles >0.25 mm in diameter, with 100% of particles < 1 mm. Organic matter content was 0.0125% by weight. Particle distribution was determined by wet sieving and organic matter determined by loss on ignition. Sand bulk density was ≈2.2 to 2.3 $g \cdot cm^{-3}$.

Dry sand in bottles was treated with flowable 52% S^o (Cleary Chemical, Somerset, N.J.) at rates of 0.0, 73.2, or 146.4 kg S^o/ha, based on the exposed surface area of the sand inside the bottle. Cross-sectional area of the bottle was ≈30 cm^2. Thus, 0.04 g material was directly applied for the 73.2-kg rate. The S^o rates were considered representative of rates conventionally applied in a turf management scheme.

After treatment with S^o, bottles were filled with either tap water, or organic carbon (OC) as sodium lactate ($C_3H_5O_3Na$) solution to exclude gaseous head space. Lactate solution was prepared as either 112 or 1120 mg lactate/kg water. The precise volume of water or lactate solution added to each bottle was unknown but was estimated to be near 35 ml. Thus, ≈13.3 or 133.3 kg lactate/ha was directly applied based on exposed surface area. Tap water or lactate solution within bottles was considered experimental solution, as ≈20 to 30 ml remained above the sand surface at all times as overlying water. Excess experimental solution was needed to facilitate measurement of dissolved and precipitated S^{2-}. Sulfate content of the tap water was 30 $\mu g \cdot ml^{-1}$ as measured by low-pressure liquid anion chromatography.

After being filled completely, units were sealed with butyl rubber stoppers, crimped with aluminum seals, then incubated at 30C for 21 days in darkness. A syringe needle stuck through the stopper served as a fluid vent to accommodate sealing. The design was completely randomized with three replications. Treatments were arranged factorially.

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Table 1. Influence of lactate and elemental sulfur on redox parameters and sulfur compounds in sand. Experimental units were incubated at 30C in darkness for 21 days. Values are means of three replications.

Treatments (kg·ha^{-1})		Redox parameters			Sulfur compounds^z (mg·kg^{-1})		
Lactate	Sulfur	pe^y	pe + pH	pH	H_2S	AS^{2-}	SO_4^{2-}
0.0	0.0	−0.5	6.9	7.4	0.0	5.1	19.1
	73.2	−2.2	4.8	7.0	4.2	46.7	27.4
	146.4	−2.1	4.8	6.9	3.8	60.2	33.6
13.3	0.0	−0.8	6.5	7.3	0.0	5.1	3.7
	73.2	−2.2	4.7	6.9	5.2	74.8	28.8
	146.4	−2.3	4.4	6.7	18.3	65.5	34.4
133	0.0	−0.8	6.2	7.0	0.7	6.1	1.1
	73.2	−1.8	5.0	6.8	2.7	86.7	22.5
	146.4	−2.6	4.0	6.6	31.2	99.7	34.6
$LSD_{P=0.05}$		0.6	0.6	0.05	9.1	17.7	4.9

^{z}Measured concentration of sulfide as free hydrogen sulfide (H_2S) or acid-soluble sulfide (AS^{2-}) or measured concentration of sulfate (SO_4^{2-}) in experimental solution.
^{y}pe = Eh(59.2) where Eh = half-cell potential for H_2.

After incubation, experimental solution from each unit was sampled for H_2S and SO_4^{2-}. For this sampling, 2 ml of solution was extracted from each closed vessel with a S-ml syringe equipped with a 0.22-µm syringe filter. Injecting 2.5 ml of O_2-free N_2 (Macy et al., 1972) into each closed unit with a second syringe forced the sample solution into the receptor syringe. Thus, filtered sample from each experimental unit was withdrawn from completely filled vessels without contact with atmospheric O_2 or interference from sand debris.

Subsamples of 0.4 ml were then subsequently drawn from the original receptor syringe with a 1-ml tuberculin syringe that was previously flushed with O_2-free N_2. To measure H_2S, the 0.4-ml subsample was injected into 4 ml moving HCl : $CuSO_4$ reagent in a 10-ml spectrophotometer cuvette according to the method of Cord-Ruwisch (1985). Solution was moved in the cuvette by using a magnetic spin bar and magnetic stir plate. Absorbance was immediately determined in a spectrophotometer at 480 nm. Reagent without sample served as a blank. The AA between blanks and water at 480 nm was zero. A calibration curve made with dilutions of sodium sulfide (Na_2S) served as a S^{2-} concentration reference. Sample SO_4^{2-} concentration was measured with remaining sample using low-pressure liquid anion chromatography.

Next, the crimp seals and butyl stoppers were removed under a constant stream of O_2-free N_2 (Macy et al., 1972) and the pe + pH (Lindsay, 1979) of remaining solution was determined. Hydrogen ion concentration was measured with a Ag/AgCl combination electrode, and pe was determined using a Pt redox electrode with a calomel reference. The term pe = Eh(59.2), where Eh = half cell potential for H_2. Addition of pe and pH constitutes a modem indication of redox potential, and both parameters are necessary to specify the redox status of aqueous environments (Lindsay, 1979).

Finally, freshly boiled tap water cooled under O_2-free N_2 was added to each bottle to refill completely. The butyl stoppers were then replaced and recrimped; a syringe needle attached to a closed S-ml syringe was used as a fluid vent. Next, 2 ml freshly boiled 37% HCl cooled under O_2-free N_2 was injected into each unit, again using the syringe assembly as a fluid vent. Vents were removed, then units were inverted several times to ensure soil contact with acidic experimental solution. The HCl volatilized any acid-soluble S^{2-} that had accumulated. Samples of experimental solution were then subsequently measured for H_2S colorimetrically via the method of Cord-Ruwisch (1985). This procedure in effect gave a measure of acid-soluble S^{2-} that had accumulated as a precipitate.

Sand in certain units had darkened by 21 days. The sand appeared to be darker where S^o or S^o plus lactate was added. Increasing the level of S^0 or S^o plus lactate intensified the darkening. The observed darkening was assumed to be due to accumulation of acid-soluble S^{2-}, possibly as FeS or MnS, which are known to be black (Atlas and Bartha, 1981). However, such was not proven in this study.

Treatment with S^o depressed ($P < 0.01$) redox potential as pe + pH in sand by lowering both pe and pH (Tables 1 and 2). Depression of pe + pH by treatment with S^o probably involved the microbial oxidation of S^o to SO_4^{2-}:

$$S^0 + 3/2\ O_2 + H_2O \longleftrightarrow H_2SO_4$$

This process should consume O_2 and increase [H+], thereby reducing redox potential. When O_2 in soils is depleted, microbial activity would be expected to release electrons (e-) to the environment, thus reducing the value of pe (Lindsay, 1979). The resulting decrease in pH due to increasing [H+] would also force a mathematical reduction in redox potential.

Lactate tended to depress pe + pH but to a much lesser extent than S^0 (Tables 1 and 2). This effect resulted primarily from a lowering of pH, as no lactate effect on pe or pe + pH was detected by analysis of variance. The effect on pH was probably due to acid production associated with degradation of the lactate. Lactate could have also increased bacterial respiration rates enhancing 0, consumption. Such does not seem unreasonable in our study, as experimental units were sealed, and O_2 diffusion was stopped. However, we did not measure O_2 consumption.

Treatment with S^o resulted in free H_2S becoming detectable and also resulted in the pronounced accumulation of acid-soluble S^{2-} (Table 1). Treatment with lactate had a much smaller effect. Lactate × S^o interactions were detected. The effect of adding lactate was to intensify the effect of S^o in producing both H_2S and acid-soluble S^{2-}. Accumulation of

Table 2. Analysis of variance summary describing the influence of sulfur and lactate on redox parameters and sulfur compounds in sand. Main effect sum of squares were partitioned into linear or quadratic components.

		F values					
		Redox parameters			Sulfur compounds^z		
Source	df	pe^y	pe + pH	pH	H_2S	AS^{2-}	SO_4^{2-}
Reps	2						
Sulfur	2	54.9**	90.1**	726.8**	27.2**	126.8**	193.4**
Linear	1	94.2**	157.6**	1057.0**	49.2**	205.8**	368.4**
Quadratic	1	15.2**	22.8**	101.0**	5.3*	47.9**	18.7**
Lactate	2	0.5^{NS}	3.2^{NS}	248.3**	6.4**	15.4**	14.3**
Linear	1	<1^{NS}	6.0*	392.0**	12.7**	30.5**	28.1**
Quadratic	1	<1^{NS}	<1^{NS}	2.0^{NS}	<1^{NS}	<1^{NS}	<1^{NS}
S × L	4	1.6^{NS}	2.1^{NS}	11.5**	6.9**	4.8**	11.7**
Error	16						

^{z}H_2S denotes free hydrogen sulfide and AS^{2-} denotes acid-soluble sulfide.
^{y}pe = Eh(59.2) where Eh = half-cell potential of H_2.
^{NS,*,**}Nonsignificant or significant at $P = 0.05$ and $= 0.01$, respectively.

S^{2-} in this study probably occurred because S^o or SO_4^{2-} was available to be reduced, adequate reducing equivalents were available, and anaerobiosis was induced. Sulfate or S^o reducing organisms were probably also present, but their populations were not documented. We assumed that the production of S^{2-} was biological in origin.

The results of this study show that adding S^o to sand depresses redox potential and stimulates production of H_2S and acid-soluble S^{2-}, especially when OC is abundant. Thus, in sand-based golf greens where S^o is applied, there is potential for inducement of anaerobiosis and subsequent production of free H_2S or acid-soluble S^{2-}. Since H_2S is a known e- transport inhibitor (Atlas and Bartha, 1981), S^o addition could contribute to a decline in turf quality. Accumulation of acid-soluble S^{2-} due to application of S^o is also consistent with the idea of black layer development as a blackening of the root zone.

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