GMO VERSUS CRISP-Cas9 GENE EDITING TOOLS TO IMPROVE HEMP GENETICS FOR PENNSYLVANIA FARMERS

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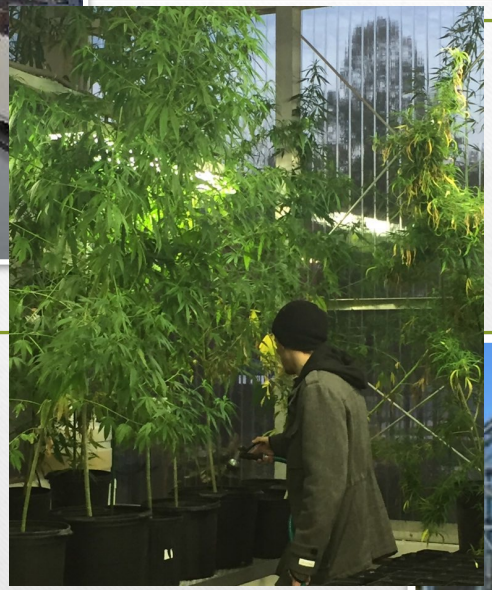

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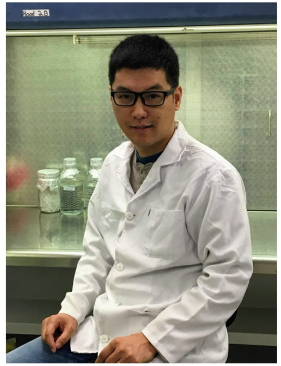

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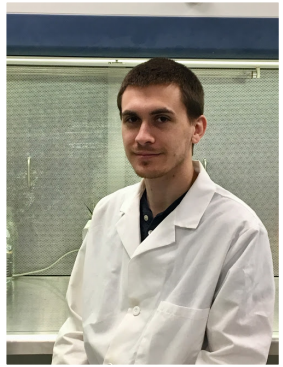
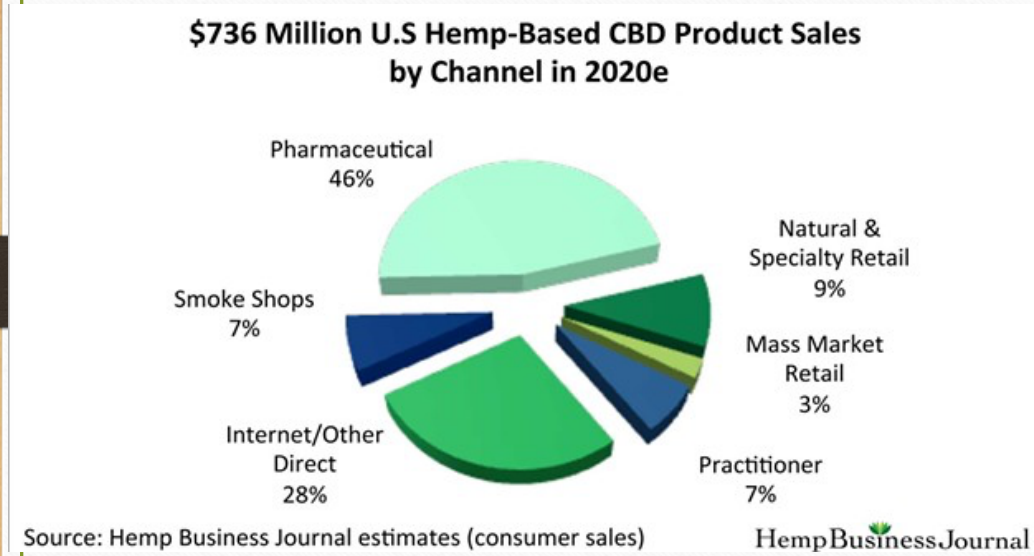
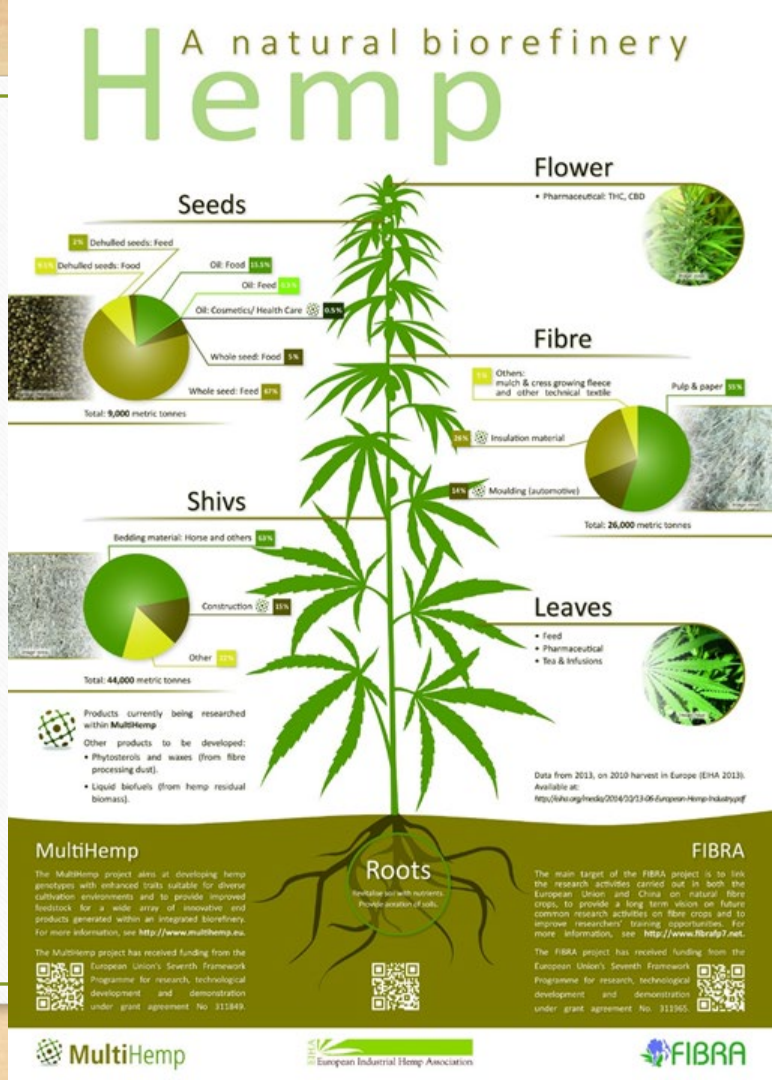
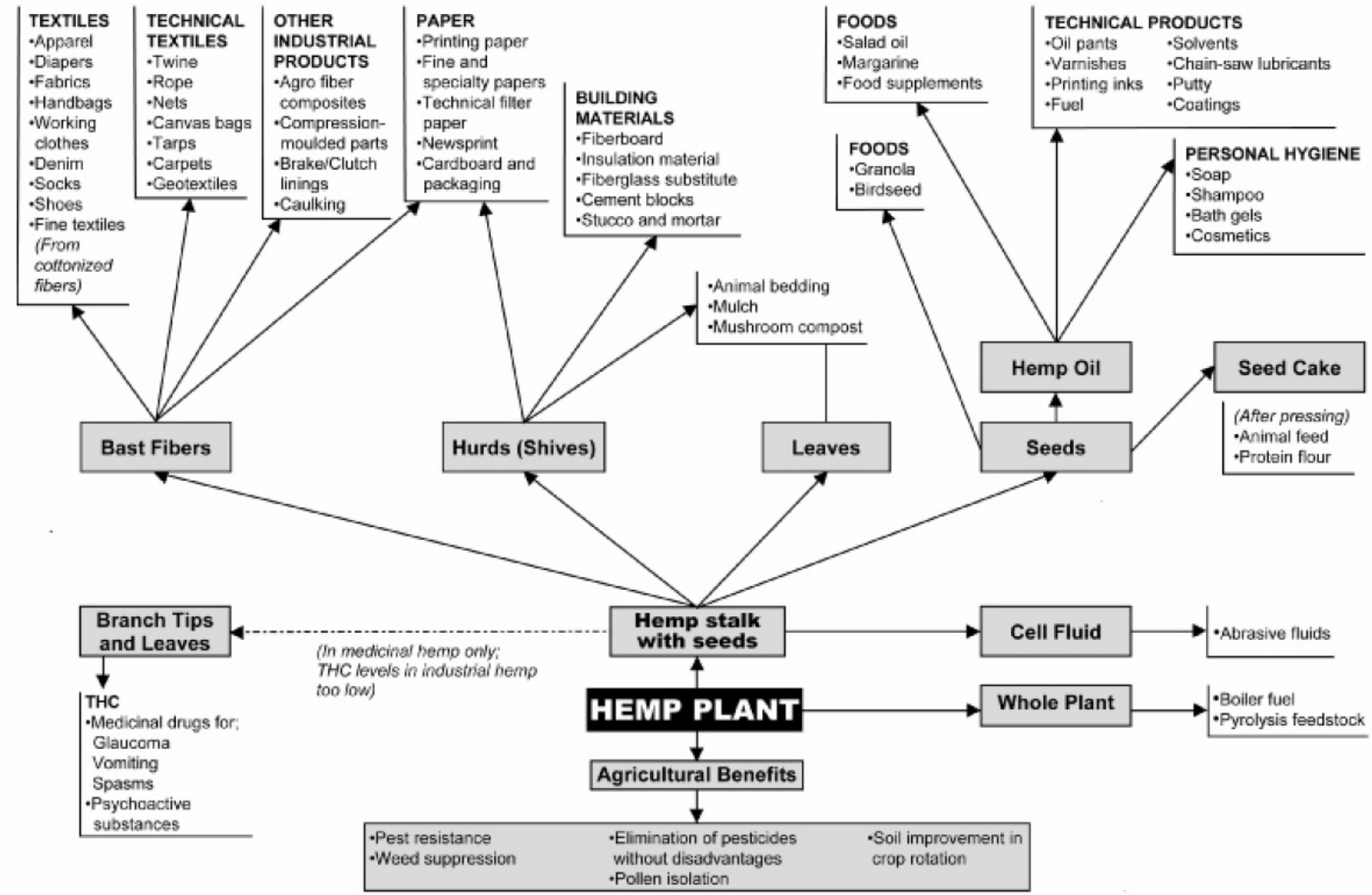


Daniel Bogush

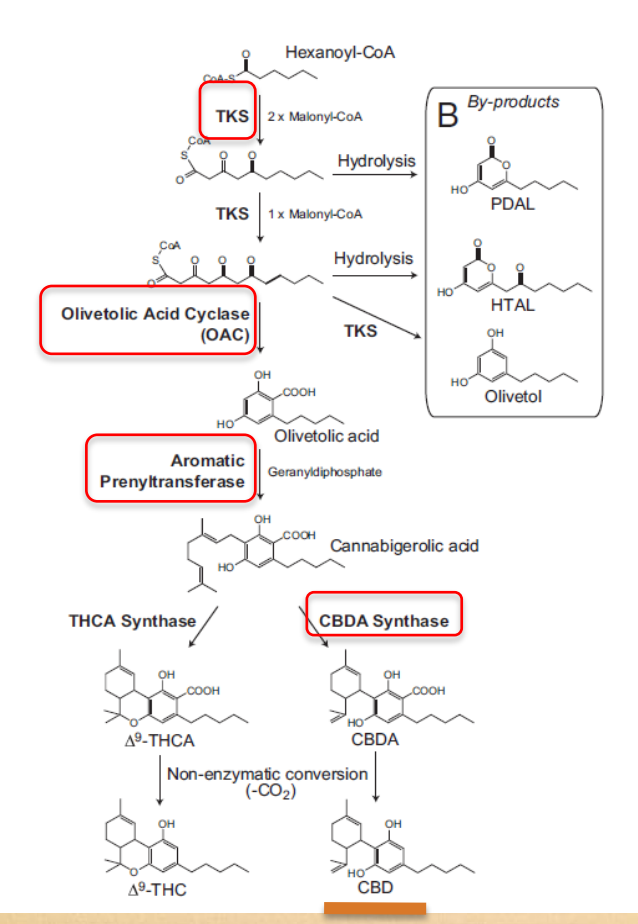
Industrial Hemp and its Applications

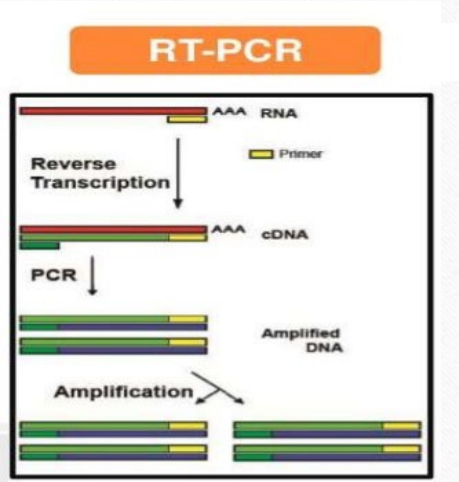


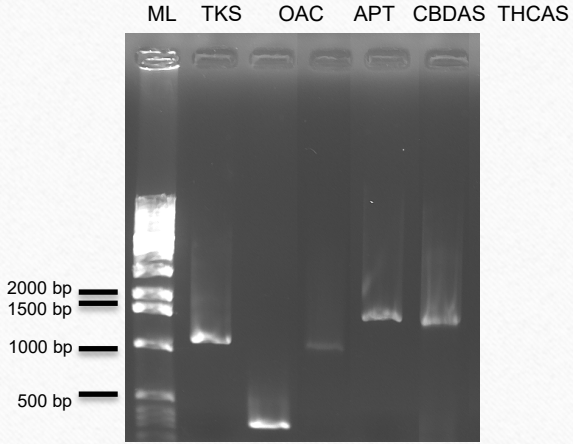



TEXTILES
•Apparel
•Diapers
•Fabrics
•Handbags
•Working clothes
•Denim
•Socks
•Shoes
•Fine textiles
(From cottonized fibers)
TECHNICAL TEXTILES
•Twine
•Rope
•Nets
•Canvas bags
•Tarps
•Carpets
•Geotextiles
OTHER INDUSTRIAL PRODUCTS
•Agro fiber composites
•Compression-moulded parts
•Brake/Clutch linings
•Caulking
PAPER
•Printing paper
•Fine and specialty papers
•Technical filter paper
•Newsprint
•Cardboard and packaging
BUILDING MATERIALS
•Fiberboard
•Insulation material
•Fiberglass substitute
•Cement blocks
•Stucco and mortar
FOODS
•Salad oil
•Margarine
•Food supplements
TECHNICAL PRODUCTS
•Oil pants
•Varnishes
•Printing inks
•Fuel
•Solvents
•Chain-saw lubricants
•Putty
•Coatings
FOODS
•Granola
•Birdseed
PERSONAL HYGIENE
•Soap
•Shampoo
•Bath gels
•Cosmetics
•Animal bedding
•Mulch
•Mushroom compost
Hemp Oil
Seed Cake
(After pressing)
•Animal feed
•Protein flour
Bast Fibers
Hurds (Shives)
Leaves
Seeds
Branch Tips and Leaves
(In medicinal hemp only; THC levels in industrial hemp too low)
Hemp stalk with seeds
Cell Fluid
•Abrasive fluids
THC
•Medicinal drugs for:
Glaucoma
Vomiting
Spasms
•Psychoactive substances
HEMP PLANT
Whole Plant
•Boiler fuel
•Pyrolysis feedstock
Agricultural Benefits
•Pest resistance
•Weed suppression
•Elimination of pesticides without disadvantages
•Pollen isolation
•Soil improvement in crop rotation

Cloning of CBD synthesis pathway genes (CBD genes)

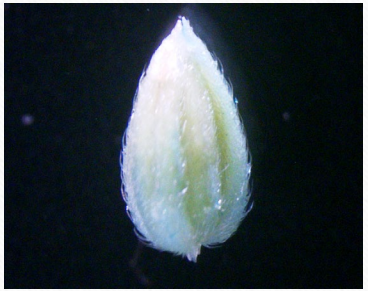






TKS: Tetraketide synthase
OAC: Olivetolic acid cyclase
APT: Aromatic Prenyltrasferase
CBDAS: Cannabidiolic acid synthase
THCAS: Δ9-tetrahydrocannabinolic acid synthase

Agroinfiltration and GUS staining of Hemp male flower

	GV3101	GUS
Loose bud clusters		
Flower bud		

An Efficient and Eco-Friendly Approach to Remediate Abandoned Mine Lands and Exploit the Potential of Industrial Hemp (*Cannabis sativa* L.)

Rabab Husain – NSF-REU

Objectives

Our **goal** was to explore the potential of industrial hemp for phytoremediation on abandoned mine land soils in Pennsylvania. In order to accomplish this goal, the following experiments were carried out:

1. Compare **seed germination** between **six varieties**, grown in **four types of soil**, under **two different environmental parameters**: inside the greenhouse and outdoors
2. Compare **plant height** and **days-to-flowering** in all treatments
3. Determine the absorption of **heavy metal uptake** by performing **soil analysis** before and after plant growth with the changes in heavy metal concentrations
4. Analyze the **changes in soil pH** before and after plant growth and correlate the differences
5. Analyze the **total cannabinoid content** in the floral buds in the mine land 1 treatment and Miracle-Gro control

Seed Germination

Table 2. Seed germination precentages after one week

Variety	Outdoor				Greenhouse			
	Miracle-Gro[a]	Pro-Mix[ac]	Mine land 1[b]	Mine land 2[b]	Miracle-Gro[a]	Pro-Mix[a]	Mine land 1[bc]	Mine land 2[b]
Fedora 17[d]	100.00±0.00	100.00±0.00	33.33±47.14	22.22±41.57	83.33±37.27	88.89±31.43	100.00±0.00	11.11±31.43
Felina 32[d]	66.67±47.14	66.67±47.14	11.11±31.43	33.33±47.14	100.00±0.00	77.78±41.57	22.22±41.57	33.33±47.14
Ferimon[d]	100.00±0.00	44.44±49.69	44.44±49.69	22.22±41.57	100.00±0.00	88.89±31.43	11.11±31.43	22.22±41.57
Futura 75[c]	83.33±37.27	77.78±41.57	22.22±41.57	33.33±47.14	66.67±47.14	66.67±47.14	77.78±41.57	44.44±49.69
Santhica 27[d]	50.00±50.00	88.89±31.43	66.67±47.14	0.00±0.00	83.33±37.27	44.44±49.69	33.33±47.14	11.11±31.43
USO 31[c]	66.67±47.14	0.00±0.00	11.11±31.43	0.00±0.00	16.67± 37.27	44.44±49.69	0.00±0.00	0.00±0.00

Average Percent (±Standard deviation)

[abcd]Letters not shared indicate a $p<0.5$

Miracle-Gro, Pro-Mix, Mineland 1, and Mineland 2 after 1 week

Conclusions

- No significant differences were observed in seed germination percentages between hemp varieties among all soil types
- Environmental parameters had no effect on the seed germination among six hemp varieties grown on Mine land 1 and Mine land 2.

Note: All Mineland 2 soil plants died shortly after germination due to low pH and high toxicity

Plant Height

Table 3. Average Plant Height at Nine Weeks

Variety	Outdoor			Greenhouse		
	Miracle-Gro[ab]	Pro-Mix[d]	Mine land 1[d]	Miracle-Gro[a]	Pro-Mix[bc]	Mine land 1[c]
Fedora 17[e]	89.00±3.00	24.33±2.05	51.00±13.00	105.75±1.25	61.00±6.68	68.67±23.33
Felina 32[e]	94.00±5.00	33.00±4.55	49.33±5.31	141.00±4.00	53.33±4.71	48.33±18.80
Ferimon[e]	94.00±3.00	16.33±13.27	39.50±30.50	84.00±20.00	75.67±21.31	58.00±26.73
Futura 75[e]	78.5±3.50	31.17±4.19	44.00±0.00	96.00±14.00	79.67±21.31	71.00±7.79
Santhica 27[e]	59.50±17.50	23.17±6.96	58.33±14.82	99.50±7.50	60.33±30.44	64.00±3.00
USO 31[e]	105.75±1.25	16.67±5.19	0.00±0.00	107.00±17.00	76.67±13.96	52.00±0.00

centimeters±standard deviation

[abcde]Letters not shared indicate a $p<0.5$

Conclusion

- Despite the high level of metal contaminants in the mine land 1 soil, plant height was comparable to the Pro-Mix soil outdoors
- Plant height of various hemp varieties grown in control soil when compared to mine land soil did not exhibit any significant differences; indicating that the presence of heavy metals did not affect the overall growth of the hemp plants

Flowering

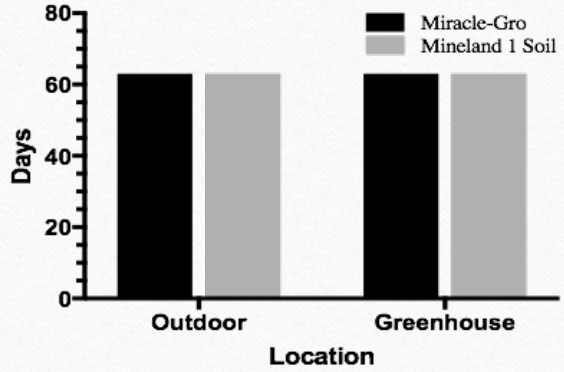


Figure 4. Days to flowering in the Fedora 17 variety

Fedora 17 indoors in Mine land 1 soil

Conclusion

Days-to-flowering were the same in Mine land 1 and Miracle-Gro soils both indoors and outdoors for variety Fedora 17

Soil Analysis

Table 4. Heavy metal content in soil at 60 days

Mine land 1											
Element	Initial	Outdoor					Greenhouse				
		Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27	Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27
As	8.65	9.6	8.98	8.97	8.77	9.09	8.19	8.91	7.89	8.11	8.41
Cd	0.34	0.39	0.39	0.34	0.35	0.38	0.28	0.36	0.37	0.32	0.32
Ni	12.83	13.96	13.77	13.64	13.51	13.34	12.88	13.47	13.31	13.27	13.26
Pb	13.74	15.94	15.22	14.07	14.4	16.4	14.01	15.45	13.74	13.51	13.78
Hg	0.043	0.046	0.056	0.046	0.045	0.046	0.048	0.046	0.046	0.045	0.049

Mg/Kg Dry Weight

Miracle-Gro											
Element	Initial	Outdoor					Greenhouse				
		Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27	Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27
As	1.75	1.04	1.21	1.29	1.25	1.15	1.49	1.86	1.53	1.39	1.25
Cd	<.25	<.25	<.25	<.25	<.25	<.25	<.25	<.25	<.25	<.25	<.25
Ni	8.62	4.08	3.81	5.39	4.82	4.93	7.27	8.14	9.67	6.78	6.07
Pb	4.18	3.69	3.96	4.52	3.95	4.13	5	5.47	4.83	4.66	4.82
Hg	0.073	0.057	<.034	<.038	0.047	0.042	0.037	0.037	0.041	0.034	0.035

Mg/Kg Dry Weight

Conclusion

- Soil analysis revealed that Mine land 1 soil had significantly higher heavy metal contaminants when compared to the Miracle-Gro control soil after plants were grown in the soil for 60 days

pH of Soil

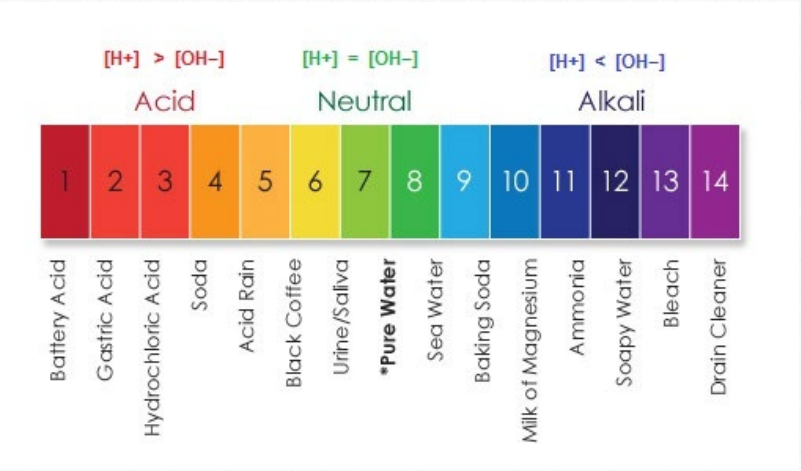


Table 5. pH in Miracle-Gro, Pro-Mix, and Mine land 1 soils initially and after 60 days

Soil	Initial	Outdoors after 60 days					Greenhouse after 60 days				
		Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27	Fedora 17	Felina 32	Ferimon	Futura 75	Santhica 27
Miracle-Gro	4.6	5.72	5.78	5.66	5.66	5.79	5.88	5.36	5.7	5.44	5.71
Pro-Mix	5.8	6.5	6.5	6.5	6.5	6.5	6	6	6	6.5	6
Mineland 1	6.7	7.09	6.68	7.19	7.2	6.54	7.01	6.91	7.21	7.3	7.13

Conclusion

The growth of all the hemp varieties used in this study grown on mine land soil caused an alteration in the soil pH thus indicating their potential for increasing the bioavailability of heavy metals to hemp

Leaf Analysis

Table 6. Metal Analysis of Felina 32

Element	Mine land 1 Outdoors	Mine land 1 Greenhouse
As	ND	ND
Cd	0.16	0.38
Pb	0.3	ND
Hg	ND	ND
Ni	1.5	0.59

Note: ND= Not Detected

Felina 32 in Mineland 1 soil

Conclusion

- There is presence of heavy metals in leaf samples collected from a 60-day-old Felina 32 variety grown in Mine land 1 soil
- The amount of Nickel content in the leaf after 60 days increased by ~2.5 times possibly indicating the release of bound Nickel into soluble form

Research Opportunities

- The genetics of hemp grain yield traits remains poorly understood.
  - Develop hemp genetics with improved shattering resistance?
  - Identify genes responsible for sex determination.
  - Creating new hemp genetics with no THC for animal feed
  - Growing industrial hemp on abandoned minelands
  - Tissue culture for elite clones.

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