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Edible Plants: The Magic Wand for Inhibition of Oxidation of Organic Compounds, Remediators of TNT and Adsorbents for Heavy Metals

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Abstract: Phytoremediation is a viable, effective, and economically attractive technology that uses plants to remove chemical contaminants from soil and groundwater. A major munitions contaminant, TNT (2,4,6-Trinitrotoluene) can be remediated by several plants such as *Myriophyllum aquaticum* (Parrot Feather), and *Catharanthus roseus*. This study focuses on screening plants that have natural antioxidant phytochemicals for their ability to remediate TNT, and heavy metals from contaminated water sources, groundwater and soil. Three kinds of bell peppers, *Capsicum frutescens* (green, red, and yellow), which contain both the antioxidant phytochemicals (carotene and vitamin C) and tomato, which also contains vitamin C, were tested to confirm their antioxidizing and remediation abilities respectively. Results for remediation abilities were analyzed by HPLC (High Performance Liquid Chromatography). Results also suggested that plants which had antioxidant properties were also able to remediate TNT and heavy metals effectively, thereby suggesting a possible correlation between antioxidant and phytoremediation abilities of the plants studied.

Key words: Phytoremediation, antioxidation, heavy metal remediation, TNT remediation.

1. Introduction

Phytoremediation is a relatively novel technology in which plants are used to degrade, remove, and treat a variety of organic and inorganic contaminants from soil, groundwater, wetlands, farming effluent, and wastewater from industrial plants [1-3]. Previous methods of remediation of contaminants from such media included excavation, incineration, adsorption, and other chemical treatments. These technologies, however, were either cost ineffective or environmentally taxing [3].

Remediation of organic compounds such as TNT (2,4,6-Trinitrotoluene), involves a variety of transformational pathways. These pathways sometimes involve various naturally occurring enzymes in the plants (such as nitroreductases), which biodegrade or reduce organic compounds to less toxic products or metabolites [4]. However, recent studies have confirmed oxidative pathways that lead to less toxic TNT metabolites [5, 6]. Results of such studies included the identification of four novel metabolites of TNT (2-amino-4, 6-dinitrobenzoic acid, 2,4-dinitro-6-hydroxybenzyl alcohol, 2-N-acetoxyamino-4, 6-dinitrobenzaldehyde, and 2,4-dinitro-6-mono hydroxytoluene) arising from plant-catalyzed oxidative metabolism including oxidation of ring-substituted methyl group and aromatic hydroxylation [5, 6].

Thus, we hypothesize that plants which contain natural antioxidizing agents will also remediate TNT from contaminated soil and water. In this case we propose that green, red, and yellow bell peppers, which are known to have two kinds of antioxidants, carotene and vitamin C (A, B, C) may be effective in the reduction of nitro groups of the TNT.

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We also propose that tomatoes, which contain vitamin C, will be able to effectively remediate TNT as well (D). To assess both antioxidant properties as well as TNT remediation ability, two sets of experiments were conducted: (1) to assess antioxidizing abilities of each of the four plant materials, and (2) to determine the TNT remediating ability of each.

TNT and its associates have also proved to be toxic to many aquatic and terrestrial species of organisms [7], including higher animals and humans, and therefore their removal from contaminated environments is especially important. Toxicity from TNT in animals includes ataxia, tremors, mild convulsions, splenic hemosiderosis, leukopenia, thrombosis, slight hepatomegaly, and kidney weight [8]. TNT toxicity to humans includes anemia, liver dysfunction, cataracts, dermatitis, leukocytosis, neurological disorders, irritation of respiratory tract (if inhaled), reduction in hemoglobin and hematocrit, and nephrotoxicity (see Appendix) [8]. No direct links to tumorigenicity in humans have been established yet, however, animal studies show an increase in urinary bladder papillomas and carcinomas in female rats as reported in the 1990 EPA (Environmental Protection Agency) report [8].

Since plants with naturally occurring antioxidant and free radical scavenging abilities have been shown to reduce cell proliferation in some types of cancer (D), and that daily intake of vitamin C has been an effective way of preventing cancer, cataracts, and muscular degeneration of the retina (E-F), it can be theorized that the intake of such foods may work against some harmful effects of TNT toxicity/mutagenicity to humans. However, this is a far- reaching adaptation of the results of the current study. There are a multitude of studies dedicated to mitigating water pollution and heavy metal remediation. However, there are a limited amount of studies covering the use of adsorbents to remediate heavy metals [9, 10], TNT [6], as well as inhibit oxidation [11]. This study focuses on using cost effective and natural adsorbents to inhibit oxidation, remediate heavy metals, and TNT.

2. Materials and Methods

2.1 Antioxidant Experiment

Of each plant, red pepper, yellow pepper, green pepper, and tomato, 50 g were placed with 100 mL of distilled water into a blender and liquefied individually. The seeds of the bell peppers were removed prior to blending. The individual mixtures were then sieved, removing the large particles, and placed in a centrifuge. The mixtures were allowed to remain in the centrifuge for 10 min until a pellet formed. The supernatant was kept and the pellet was discarded. Of the supernatants, 70 mL were placed in a glass container along with a magnetic stirring rod and set on a magnetic stirrer. Acetophenone (5 mL), and bleach (70 mL) were added to the supernatant. A control was also set up in a similar manner, which included distilled water in place of the supernatant. The mixtures and control were allowed to stir for 45 min. After the stirring period, 0.5 g of sodium sulfite was placed into each reaction jar (including the control) to kill any unreacted bleach. The mixtures were then transferred to a separatory funnel. The mixtures were washed twice with 50 mL of diethyl ether in order to achieve an organic layer and aqueous layer. Once the organic layer was obtained, the four layers from each plant were placed on an ice bath and concentrated HCl was added dropwise to each in order to protonate the mixture. The ice served to speed up the precipitation process. After several drops of HCl were added, any precipitate that formed was filtered by Hirsch filtration, dried, and weighed.

2.2 Phytoremediation of TNT Experiment

The supernatants (20 mL) of each plant were subjected to 20 mL TNT solution at a concentration of 20 ppm, and a control was set up under the same condition without the inclusion of any plant material. Instead of plant material, the control used distilled water. Aliquots of the mixture were then taken at various time intervals (0 h, 22 h, 52 h), and filtered to remove any plant material. Each aliquot sample was analyzed using HPLC (High Performance Liquid Chromatography).

2.3 Phytoremediation of Heavy Metals

Standard solutions of 1,000 ppm of calcium, copper, magnesium, and zinc 2+ ions were prepared by dissolving equivalent amounts of the corresponding salts in 1,000 mL of solution. Triplicate samples of 40 mL of the contaminated solutions were transferred into separate centrifuge tubes. Each sample was treated with 5 g of plant substrate. The samples were vortexed to mix, and agitated for 22 h at room temperature. After agitation, each sample was centrifuged at 3,000 rpm for 10 min. The supernatant was decanted and analyzed for residual metal ion concentration using the EPA method 6010 (ICPAES (Inductively Coupled Plasma-Atomic Emission Spectrometry)).

3. Results

3.1 Antioxidant Experiment

The control of the experiment lacking plant supernatant yielded a precipitate of 1.91 g, while the red, yellow, and green pepper, as well as the tomato did not yield any detectable product (Figs. 1 and 2).

Fig. 1 Green and red bell pepper antioxidant experiment results.

Fig. 2 Yellow bell pepper and tomatoes antioxidant results.

3.2 Phytoremediation of TNT Experiment

The results of the TNT remediation experiment can be viewed in Figs. 3-6. At 22 h, the yellow bell pepper appeared to be most effective in its removal of TNT from the solution with a HPLC area of 0.86 remaining in the solution, whereas, red pepper left 2.48, green pepper left an area of 1.23, tomato left 4.08, and the control that did not include plant material left 17.51. At 52 h, the yellow pepper left an area of 0.19, red pepper left 0.23, green pepper left 1.3, tomato left 0.69, and the control left 20.32.

3.3 Phytoremediation of Heavy Metals

The results of the heavy metal remediation experiment can be found in Figs. 9-13. After 22 h, the residual concentrations of metal were collected and compared to the standard concentrations of heavy metal aqueous solutions. All fruits and vegetables extracts adsorbed almost half of the metal contaminants in the various aqueous solutions. The standard concentration for calcium was about 995 ppm. The order of average residual metal concentration for calcium is: (green bell pepper 471 ppm < tomatoes 475 ppm < yellow bell pepper 484.5 ppm < red bell pepper 497.5 ppm). The standard concentration for copper was about 2,070 ppm. The order of average residual metal concentration for copper is: (yellow bell pepper 961.5 ppm < red bell pepper 1,003.5 ppm < tomatoes 1,013.5 ppm < green bell pepper 1,095 ppm). The standard concentration for magnesium was about 966.5 ppm. The order of average residual metal concentration for magnesium is: (tomatoes 530 ppm < green bell pepper 557.5 ppm < yellow bell pepper 572.5 ppm < red bell pepper 575.5 ppm). The standard concentration for zinc was about 1,105 ppm. The order of residual metal concentration for zinc is: (tomatoes 513.5 ppm < red bell pepper 542.5 ppm < green bell pepper 553 ppm < yellow bell pepper 556 ppm).

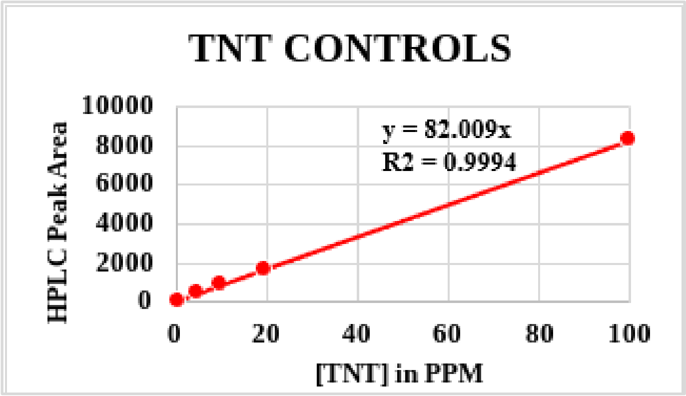

Fig 3 TNT calibration curve.

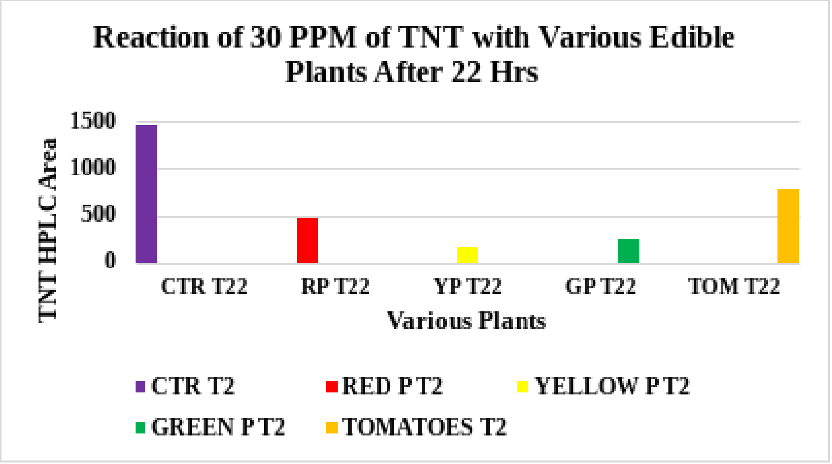

Fig. 4 Reaction of 30 ppm of TNT with various edible plants after 22 h.

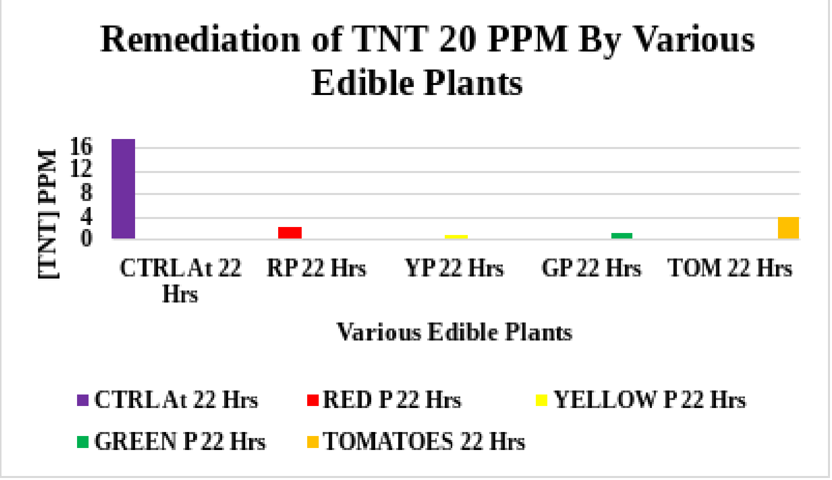

Fig. 5 Remediation of TNT 20 ppm by various edible plants.

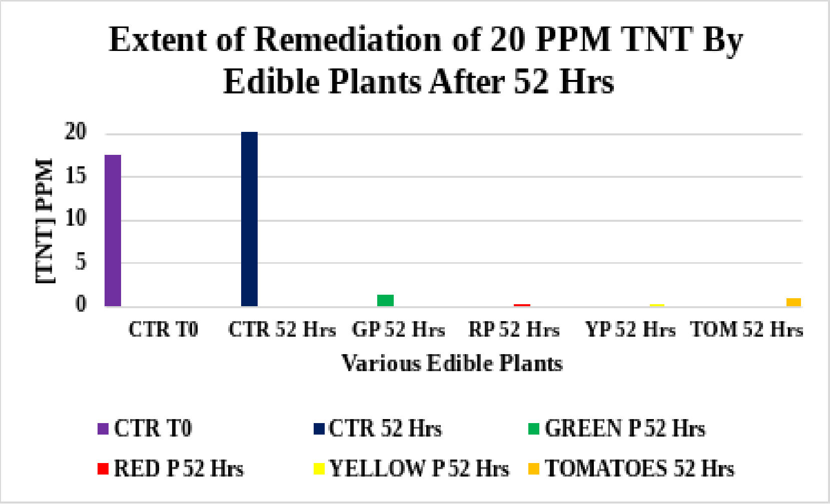

Fig. 6 Extent of remediation of TNT 20 ppm by various edible plants.

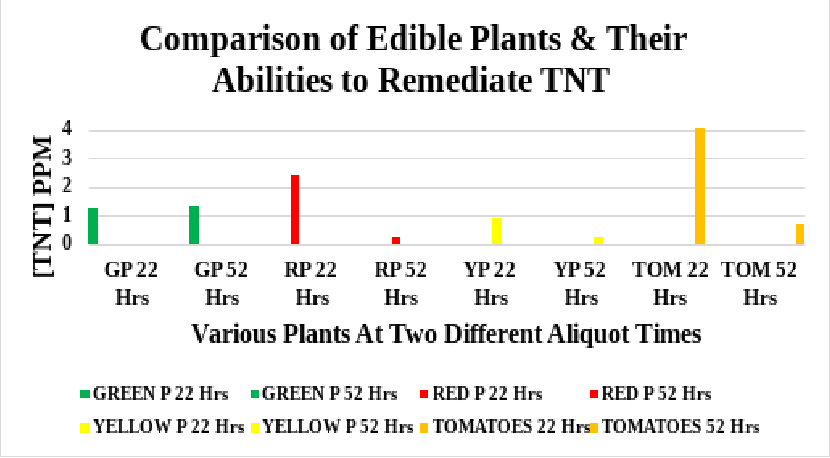

Fig. 7 Comparison of edible plants & their abilities to remediate TNT.

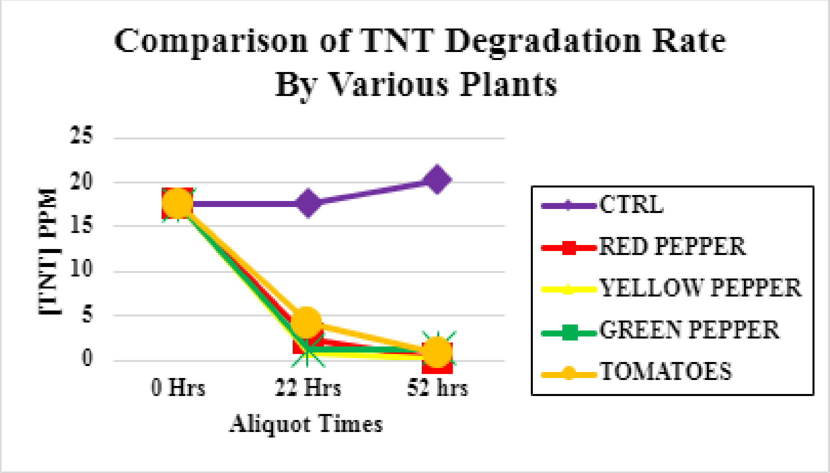

Fig. 8 Comparison of TNT degradation rate by various plants.

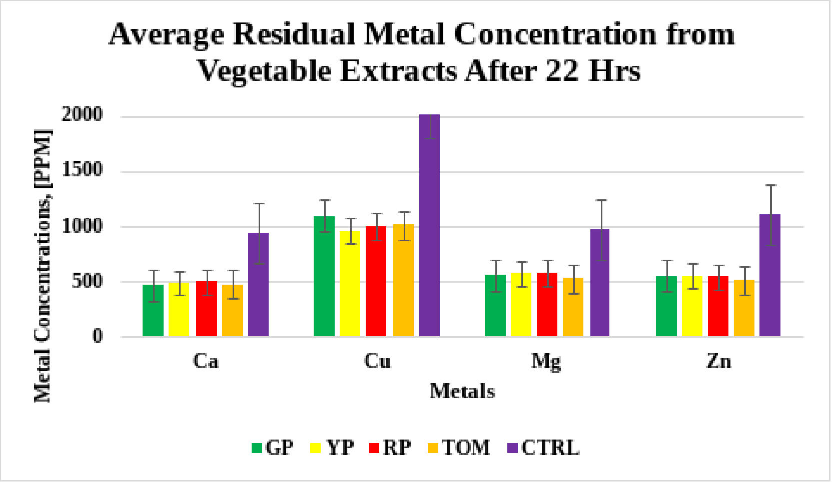

Fig. 9 Average residual metal concentration from vegetable extracts after 22 h.

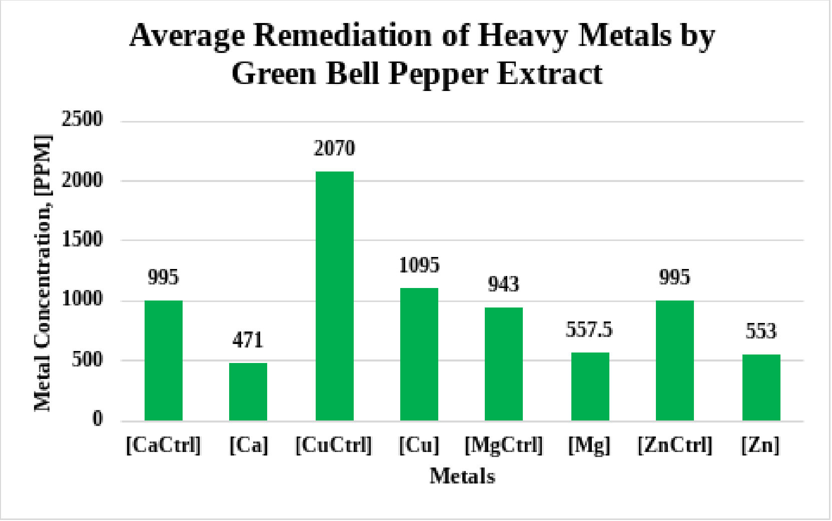

Fig. 10 Average remediation of heavy metals by green bell pepper extract.

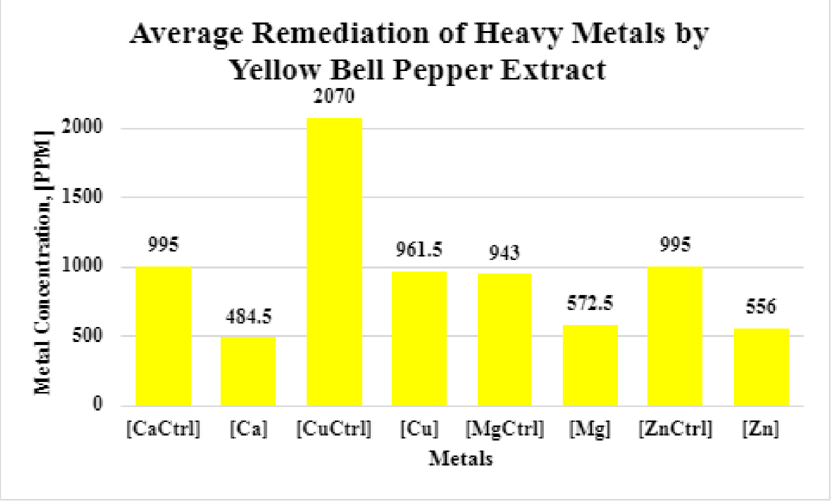

Fig. 11 Average remediation of heavy metals by yellow bell pepper extract.

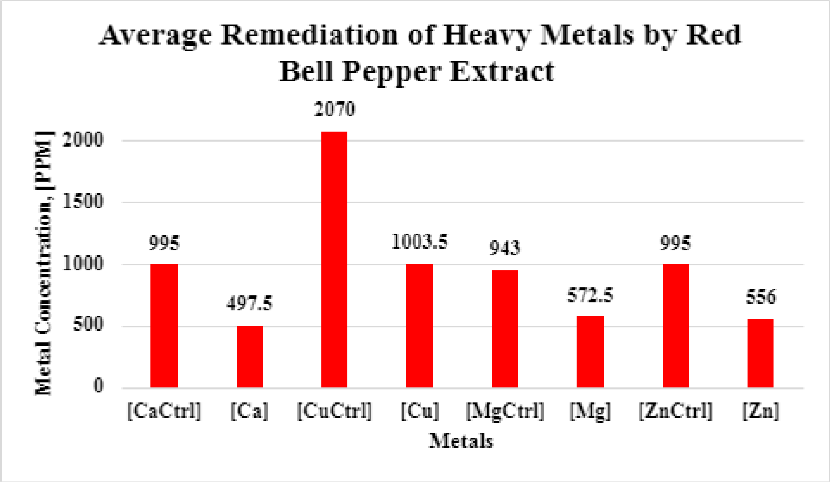

Fig. 12 Average remediation of heavy metals by red bell pepper extract.

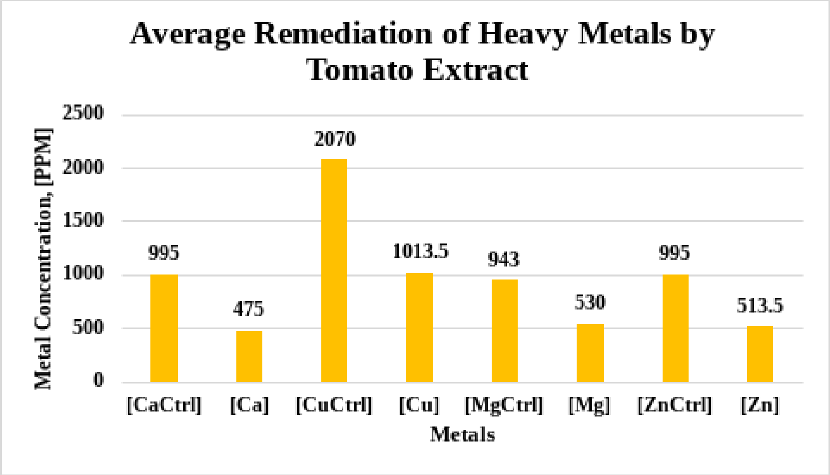

Fig. 13 Average remediation of heavy metals by tomato extract.

4. Discussion

4.1 Antioxidant Experiment

When acetophenone was oxidized, a benzoic acid was formed. However, when similar oxidation reaction of benzophenone was carried out in the presence of an antioxidant, no benzoic acid is formed. Thus, the control experiment produced benzoic acid, whereas in the presence of plant biomaterial with antioxidant properties, no benzoic acid product was observed. It is worthy to note that all of the plants tested inhibited oxidation relative to the control (oxidation = 1.91 g).

4.2 Phytoremediation of TNT Experiment

All five plants tested, removed or remediated various amounts of the total TNT used in the experiment. Specifically, yellow pepper appeared to remove the most TNT at each time interval. The rate of removal of the TNT in solution varied from plant to plant. The red pepper and tomato appeared to have a faster rate of TNT remediation from 22 h to 52 h, as is seen by the drastic reduction in area from 22 h to 52 h, while the rate of the reaction in green pepper and yellow pepper appeared to be more gradual. The discrepancy between the area of the control at 22 h and 52 h is due to water lost from solution through evaporation.

All four plants tested inhibited oxidation and remediated the tested amount of TNT. Therefore, the results suggest a possible correlation between the anti-oxidizing ability of the plants and their remediation ability of TNT. However, to make a stronger correlation, many more plant types need to be tested using similar methods.

Also, to improve upon this experiment, aliquots in the TNT experiment need to be taken at shorter time intervals.

4.3 Phytoremediation of Heavy Metals

Four of the five plants were tested as adsorbents for heavy metals. After 22 h, the fruits and vegetables remediated almost half of the heavy metals from the all contaminated aqueous solutions. Therefore, the results suggest that the fruit and vegetable adsorbents seem to be completely saturated with heavy metals around 450 ppm. Thus, the fruit or vegetable that remediated the most metal varies in each set of samples. In order to make a stronger correlation, a study with lower concentrations of heavy metals samples needs to be tested with the same method.

5. Conclusion

Although there are many research studies that are dedicated to the use of plants in the remediation of heavy metals and TNT; however, many of them do not examine the use of the same plants for multiple purposes. The research presented in this paper analyzes the use of plant extracts in the phytoremediation of heavy metals, TNT, as well as inhibition of oxidation. The green bell pepper, yellow bell pepper, red bell pepper, and tomatoes used in this research demonstrate an ability to remediate TNT and toxic metals from contaminated aqueous solutions. The results presented in the anti-oxidation experiment indicate that all the plants tested effectively inhibited the oxidation of acetophenone. Thus, green bell pepper, yellow bell pepper, red bell pepper, and tomatoes can be used for multiple purposes in chemical research.

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References

[1] Bhadra, R., Spanggord, R. J., Wayment, D. G., Hughes, J. B., and Shanks, J. V. 1999. "Characterization of Oxidation Products of TNT Metabolism in Aquatic Phytoremediation Systems of Myriophyllum Aquaticum." *Environ Sci Technol.* 33: 3354-61.

[2] Medina, F. V., Rivera, R., Larson, L. S., and McCutcheon, S. 1998. "Phytoreactors Show Promise in Treating Munitions Contaminants." *Soil and Groundwater Cleanup* 20: 19-24.

[3] Medina, F. V. Phytoremediation Information Sheet. http://www.tricity.wsu.edu/~vmedina/phyt_gen.html.
Van Dillewijn P, Couselo JL, Corredoira E, Delgado A, Wittich RM, et al. "Bioremediation of 2,4,6-trinitrotoluene by Bacterial Nitroreductase Expressing TransgenicAaspen." *Environ. Sci. Technol.* 2008 42:7405–7410.

[4] Bhadra, R., Wayment, D. G., Hughes, J. B., and Shanks, J. V. 1999. "Conformation of Conjugation Processes during TNT Metabolism by Axenic Plant Roots." *Environ Sci Technol.* 33: 446-52.

[5] Victor F. Medina, Steven Larson, Lovell Agwaramgbo, Waleska Perez. "Treatment of Munitions using Phytoslurries." *Int. J. Phytoremediation* 2002 4 (2): 143-56..

[6] Kaplan, D. L., and Kaplan, A. M. 1982. "Mutagenicity of 2,4,6-Trinitrotoluene-Sufactant Complexes." *Bull Environm Contam Toxicol.* 28: 33-8.

[7] Opresko, D. M., and Chemical Hazard Evaluation Group. 1996. *Toxicity Summary for 2,4,6-Trinitrotoluene: Executive Summary*.

[8] Lovell Agwaramgbo, Christopher Alisa, and Jailen Doyle "Examining Adsorbent Charge Effect on Metal Removal from Contaminated Water." *Journal of Environmental Science and Engineering* A 2022 (11): 201-205.

[9] Lovell Agwaramgbo, Jailen Doyle, Corneisja Harrison, Honour Williams; Comparative Evaluation of Calcium, Magnesium. "Copper and Zinc Removal by Wood Ash, Sodium Carbonate and Sodium Hydrogen Phosphate." *Journal of Environmental Protection* 2021 (12): 454-461.

[10] Lovell Agwaramgbo, Tishina Okegbe, Tajeve Wright, Stephen Igwe, and Victor Ogburie. "Inhibition of the Oxidation of Acetophenone by Aqueous Extracts of Edible Fruits and Vegetables." *Journal of Modern Chemistry and Applications* 2013 Vol. 1 #105: pp 1-3.

Appendix

Ataxia: Lack of coordination

Hemosiderosis: Chronic iron overload in tissue

Leukopenia: Reduced number of lymphocytes, monocytes, eosinophils, or basophils.

Thrombosis: Formation of a blood clot in the heart or blood vessel.

Hepatomegaly: Asymptomatic liver enlargement resulting from numerous types of hereditary enzyme deficiencies.

Leukocytosis: Increase in white blood cell count as response to noxious stimuli; part of inflammatory reaction.

Nephrotoxin: Is a toxic agent/substance that inhibits, damages, or destroys the cells or other tissue of the kidneys.