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Phytoremediation potential of *Cannabis sativa* and *Argemone maxicana* at industrial discharge sites in Moradabad U. P.

Prashant Kumar
Department of Botany, Hindu College, Moradabad, Uttar Pradesh, India
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Abstract
The concentrations of nine metals magnesium (Mg), cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Nickel (Ni), Lead (Pb) and Zinc (Zn) in soils and natural herb plant of *Cannabis sativa* and *Argemone maxicana* collected from industrial discharge sites were investigated. The aim was to define that which species exhibit the greatest accumulation and evaluate whether these species could be usefully employed for phytoremediation. The bioaccumulation and transfer of metals from soil to plants was evaluated in terms of bioconcentration factor (BCF). Results showed that the value of BCFs, in both *Cannabis sativa* and *Argemone maxicana* was high. Many plants of these species from three different sites were screened for Cd, Cu, Ni, Co, Pb and Zn, and found that plants from different sites were efficient to take up and translocate more than one heavy metal from root to shoot but in different ratio. According to accumulation capability for most metals, both *Cannabis sativa* and *Argemone maxicana* were found to be suitable for phytoremediation programs of industrial polluted soils.

Keywords: Heavy metals, pollution, phytoremediation, bioconcentration factor

Introduction

In the modern world the large-scale industrialization and production of a variety of chemical compounds have led to global deterioration of the environmental quality (Chakravarty *et al,* 2010) [12]. In India, the pollution of main cities has increased in the past few decades because of increase in population and industrial activities that is likely to increase the volume of pollutants discharged to that area. The treatment of this waste water before disposal is regulatory; however, owing to the high effluent treatment costs and due to elaborate physico-chemical methods, partial treatment is carried out and much quantities of this effluent is sometimes stored in lagoons, or let out into the surface water bodies or streams or in unlined tanks, thus adversely affecting the natural water resources and environment (Karanam & Joshi, 2010) [25]. Metal persistence in soil for much longer periods than in other compartments of the biosphere is a matter of serious concern. According to Beyermann and Hartwig (2008) [7] heavy metals like Cd, Cr, Ni, Pb, etc, has classified to be carcinogenic to human and wildlife. Numerous efforts have been undertaken recently to find cost-effective technologies for remediation of heavy metal-contaminated soil (Chatterjee *et al,* 2011) [14]. Therefore, plants can be used to cleanup heavy metal pollutants from the soil. This cost-effective approach is called phytoremediation which also referred as green solution (Butcher, D.J, 2009) [10]. Phytoremediation has recently become a subject of public and scientific interest and a topic of many researches. The natural vegetation plays an important ecological role for chemically polluted soil (Antonkiewicz and Jasiewicz, 2002) [3].

Several authors include the bioaccumulation factor (BAF or BCF) as an element for classification as a hyperaccumulator species. According to Antonsiewicz *et al,* (2008) and Yoon *et al,* (2006) [4, 45], native plants should be preferred for phytoremediation because these plants are often better in terms of survival, growth and reproduction under environmental stress in comparison to plants introduced from other environment. Therefore, the search for native plants that are tolerant to heavy metals is of particular importance. Few studies evaluated the potential for phytoremediation of native plants (Ginocchio and Baker, 2004) [21] under field conditions. Khairia M. Al-Qahtani (2012) [26] showed that the concentrations of heavy metals in the soils have the sequence of (Fe > Zn >Cr >Cu > Pb > Ni > Co > Cd) while in plants (*Calotropis procera*, *Citrullus colocynthis*, *Rhazya stricta*, *Cassia italic*, *Phragmite australis*, *Cyperus laevigatus* and *Argemone maxicana*) the trend was (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd). With this idea, and public concern over soil contamination by heavy metals in industrialized area in Riyadh City, Saudi Arabia, searching for plant species with the potential for phytoremediation is necessary because no metal-tolerant and metal hyperaccumulator plants with potential application to this area have been reported. Therefore, two aims of this study were: first to evaluate the concentrations of Cd, Cr, Cu, Fe, Pb, Ni and Zn in soils and two plant species (*Cannabis sativa* and *Argemone maxicana)* and second; to evaluate whether these species could be usefully employed in phytoremediation.

Materials and Methods

Study Site & Sampling

Moradabad is situated on the bank of river Ram Ganga (a tributary of holy Ganga) at a distance of 167 km from national capital New Delhi. District Moradabad lies between 280 21’ to 280 16’north latitude and 780 4’ to 790 east longitude. This district occupies an area of 3493 km^2. It is bounded by Rampur in east, Amroha in west, Bijnor and Nainital in north and Budaun in south. The climate is subtropical having three seasons, rainy (July-October), winter (November-February) and summer (March-June).

The most important industries in this area are: food industries, metal industries (especially brass) and agro-based industries such as paper mills, distilleries etc. Soil samples were collected at nearby discharge outlet from three sites of

Delhi Road, Sambhal Road and Kanth Road industry of district Moradabad separately at 10cm, 20cm & 30cm depth. Soil samples of each site with same depth were mixed and replicates were used.

The plant sampling was done around each industrial discharge site. Three plants of the species were selected at a time from each industry and the mean of three observations was considered as final. The samples were dried for nearly 48 hours at 85°C in an oven and the reduced to powder in electric grinder. Then they were kept in polythene bags to avoid any loss in fresh weight.

Heavy Metal Analysis

Heavy metals were then analyzed by direct aspiration of the soil and plant sample solution into an Atomic Absorption Spectrophotometer. The Bioconcentration Factor (BCF) was calculated as metal concentration ratio of plant root to soil as given in the following equation-

BCF = Metals in plant/Metals in soil

Results and Discussion

The top soils from different sampling sites had small differences in texture and pH in the area under investigation. The concentration of metals decreases at increasing depth of soil. These metals are present in spent wash as a result of smelting of metalliferous ores, application of fertilizers, pesticides & municipal wastes. The results revealed that all sites are characterized by loam and sandy texture. As indicated by Kumar *et al,* (2011) [27] the pH of soil was acidic to slight alkaline in nature and varies from 6.6-7.6.

Heavy metals contamination of soil showed several problems, including phytotoxic effects of certain elements such as Cd, Pb, Zn and Cu (known as micronutrients) and these cause several phytotoxicities if critical endogenous levels are exceeded (Susarla *et al,* 2002, Chehregani *et al,* 2005) [15, 39]. The up taking of harmful elements through food or forage plant species is a more serious problem because being transferred to the food chain and, finally to human beings. All the heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants. Currently an emerging technology is the use of plants for environmental restoration. In this approach, plants capable of accumulating high levels of metals are grown in contaminated soils (Lasat, 2002) [29]. Interest in phytoextraction has significantly grown following the identification of metal accumulator plants.

Table 1: Metal composition in soil with *Cannabis sativa* and *Argemone maxicana* as dominant species at industrial discharge site in relevant seasons

Site	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DRS	2.976	-	1.985	27.820	23.083	8.208	0.238	172.407	0.790
KRS	2.906	0.521	2.557	29.269	27.520	10.656	0.299	188.952	0.724
SRS	2.998	-	-	27.432	24.996	9.568	0.325	113.891	0.654
DRW	2.588	-	1.972	25.332	22.632	6.666	0.168	154.785	0.748
KRW	2.632	0.416	1.956	25.868	25.810	8.961	0.186	180.837	0.536
SRW	2.870	-	-	25.516	24.573	6.956	0.316	111.045	0.530

D=Delhi Road, KR=Kanth Road, SR=Sambhal Road, S= Summer, W=Winter

Plant and soil analysis revealed that the accumulation is considerably the consequence of a kind of elements (Dermirezen, 2002). Kumar *et al,* (2011) [18, 27] concluded that average total N_2 was maximum (1226.751 ppm) in upper part up-to 10 cm depth whereas lowest average value (806.993 ppm) was at 30 cm depth around distillery discharge. The average value for available phosphorus ranges from 1.836 to 4.895 ppm. *Cannabis sativa* was found dominant in summer season at all the sites. This plant shows highest concentration of available Mg (240.481 ppm) followed by Fe (39.833 ppm), Mn (38.550 ppm), Zn (18.102 ppm), Cu (5.410 ppm), Lead (4.950 ppm), Cd (1.009 ppm) and Ni (1.229 ppm) with Bioconcentration factor of 1.395, 1.432, 1.670, 2.205, 1.818, 2.494, 4.239 and 1.556 respectively at Delhi Road industry site. *Argemone maxicana* has highest concentration of available Mg (221.819 ppm) followed by Fe (34.059 ppm), Mn (30.241 ppm), Zn (15.196 ppm), Cu (4.556 ppm), Lead (3.473 ppm), Ni (0.991 ppm) and Cadmium (0.788) with Bioconcentration factor of 1.433, 1.345, 1.336, 2.280, 1.760, 1.761, 1.325 and 4.690 respectively in winter season at this site. Thus, *Argemone maxicana* also has highest Bioconcentration factor for cadmium. The Bioconcentration factor in *Argemone maxicana* for Cd is followed by zinc and lead.

During winter season at Kanth Road industry *Argemone maxicana* has highest concentration of available Mg (255.380 ppm) followed by Fe (36.270 ppm), Mn (34.716 ppm), Zn (17.024 ppm), Cu (5.586 ppm), Pb (3.994 ppm), Cr (1.841 ppm), Ni (1.450 ppm) and Cd (1.053 ppm) with Bioconcentration factor of 1.412, 1.402, 1.345, 1.900, 2.123, 2.042, 4.425, 2,705 and 5.661 respectively. During summer season at Kanth Road industry *Cannabis sativa* has highest concentration of available Mg (277.61 ppm) followed by Mn (41.695 ppm), Fe (42.656 ppm), Zn (22.502 ppm), Cu (6.195 ppm), Pb (4.775 ppm), Cr (1.318 ppm), Ni (1.655 ppm) and Cd (1.248 ppm) with Bioconcentration factor 1.469, 1.515, 1.457, 2.112, 2.132, 1.867, 2.530, 2.286 and 4.134 respectively.

Argemone maxicana has highest concentration of available Mg (173.273 ppm) followed by Fe (38.882 ppm), Mn (32.613 ppm), Zn (13.818 ppm), Cu (5.122 ppm), Ni (0.957 ppm) and Cd (0.715 ppm) with Bioconcentration factor 1.560, 1.524, 1.327, 1.985, 1.785, 1.806 and 2.263 respectively at Sambhal Road industries in winter. During summer season *Cannabis sativa* was found dominant at Sambhal Road industry with highest concentration of available Mg (187.142 ppm) followed by Fe (41.795 ppm), Mn (39.223 ppm), Zn (18.817 ppm), Cu (5.557 ppm), Ni (1.273 ppm) and Cd (0.994 ppm) with Bioconcentration factor of 1.643, 1.524, 1.569, 1.967, 1.854, 1.947 and 3.058 respectively. This plant has highest Bioconcentration factor for Cd (3.058) followed by Zn (1.967) at this site.

The results of Malik *et al,* (2010) [33] showed that most of plant species accumulated higher concentration of Pb, Cu and Zn than normal limits in shoots. The concentrations of the investigated heavy metals in soil dominated with *Cannabis sativa* possess the sequence of (Fe > Zn >Cr >Cu > Pb > Ni >Co > Cd) while in plants the trend was (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd). However, the concentrations of the investigated heavy metals in soil dominated with *Argemone maxicana* possess the sequence like Fe > Zn >Cr >Cu > Pb > Ni > Co > Cd while in plants the trend was slightly different (Fe > Zn >Cu >Cr > Ni > Co > Pb > Cd).

A toxic element Cadmium (Cd) exists along with Zn in the nature. Generally, The Cd concentration in the soils was relatively low. The highest Cd concentration was recorded at associated with *Argemone maxicana.* This may be attributed to the relatively high pH value which enhances Cd precipitation at this site (El-Rayis and El-Sabrouti, 1997) [19]. The highest uptake of Cd was attained by *Calotropis procera* stem followed by *Argemone maxicana*.

Table 2: Metal compositions in *Cannabis sativa* and *Argemone maxicana* at industrial discharged sites during relevant seasons

Site	Dominant species	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DRS	*C. sativa*	5.410	-	4.950	39.833	38.550	18.102	1.009	240.481	1.229
KRS	*C. sativa*	6.195	1.318	4.775	42.656	41.695	22.502	1.248	277.61	1.655
SRS	*C. sativa*	5.557	-	-	41.795	39.223	18.817	0.994	187.142	1.273
DRW	*A. maxicana*	4.556	-	3.473	34.059	30.241	15.196	0.788	221.819	0.991
KRW	*A. maxicana*	5.586	1.841	3.994	36.270	34.716	17.024	1.053	255.38	1.450
SRW	*A. maxicana*	5.122	-	-	38.882	32.613	13.818	0.715	173.273	0.957

DR=Delhi Road, CR=Kanth Road, SR=Sambhal Road, S= Summer, W=Winter

Table 3: Bioconcentration factor in *Cannabis sativa* and *Argemone maxicana* at industrial discharge sites during relevant seasons

Site	Dominant species	Cu	Cr	Pb	Fe	Mn	Zn	Cd	Mg	Ni
DRS	*C. sativa*	1.818	-	2.494	1.432	1.670	2.205	4.239	1.395	1.556
KRS	*C. sativa*	2.132	2.530	1.867	1.457	1.515	2.112	4.134	1.469	2.286
SRS	*C. sativa*	1.854	-	-	1.524	1.569	1.967	3.058	1.643	1.947
DRW	*A. maxicana*	1.760	-	1.761	1.345	1.336	2.280	4.690	1.433	1.325
KRW	*A. maxicana*	2.123	4.425	2.042	1.402	1.345	1.900	5.661	1.412	2.705
SRW	*A. maxicana*	1.785	-	-	1.524	1.327	1.985	2.263	1.560	1.806

DR=Delhi Road, CR= Kanth Road, SR=Sambhal Road, S= Summer, W=Winter

Chromium (Cr) is a non-essential metal to plant growth, and may be possible that plants do not have any specific mechanism and transport of Cr (Shanker *et al,* 2005) [37]. Generally, soils of all selected sites in the area under investigation acquired absence of Cr except Kanth Road industrial discharge site associated with *Cannabis sativa* and *Argemone maxicana*. Results from the present study showed that roots of all plants attained higher Cr concentrations than other organs, with the highest value of 628.8 528 ug/g d.w attained by *Phragmite australis* root. This could be because Cr is immobilized in the vacuoles of the root cells and showed less translocation, thus rendering it less toxic. This may be a neutral toxicity response of the plants (Macnicol and Beckett, 1985) [32]. According to him the toxic levels of Cr in plants range from 1 to 10 µg/g dry weight.

Although Copper (Cu) is an essential element for plants and animals but, excessive concentrations are considered to be highly toxic. Cu concentrations in plants above 10-30 µg/g d.w are regarded as poisonous (Macnicol and Beckett, 1985) [32]. Iron (Fe) is an essential micronutrient for plants and animals (Kunze *et al,* 2001) [28]. However, excessive Fe uptake can produce toxic effects. Fe is the most abundant metal in the studied area. The highest Fe concentration was determined in the soil of Kanth Road site, affected by industrial discharges from a nearby industrial complex. The results obtained from plant analysis asserted that roots of both plants are found to be highly capable of metal accumulation. According to Allen (1989) [2], Fe concentrations above 40-500 µg/g d.w are considered as toxic to plants. The ability of roots to reduce Fe^{+3} to Fe^{+2} is believed to be fundamental in the absorption of this cation by most plants (Tinker, 1981) [41]. Moreover, some bacterial species (like *Metallogenium* sp.) are involved in reduction of iron and are known to accumulate iron on the surface of living cells (Weinberg, 1977) [43]. Higher concentrations of Fe in the roots of the investigated species could be due to its precipitation in iron- plaque on the root surface (Tanner, 1996 and Batty *et al,* 2002) [6, 40].

Lead (Pb) is the least mobile among the heavy metals. It is not essential but toxic to plants. Recent results of Pb translocation and uptake studies showed that Pb is mobile within the plant under certain conditions (Meers *et al,* 2005). Also, Blaylock and Huang (2000) [8, 34] indicated that shoot Pb concentrations reached a value similar to the concentration found in intact roots of the same species, when it is immersed in a nutrient solution containing Pb. Generally, Pb concentrations in both plants were notably higher. This could be related to airborne Pb deposition emitted from a heavily traffic high way affected the open area under investigation. According to Ross (1994) [36], 30-300 µg/g Pb concentrations are considered toxic to plants. Plants with higher Pb translocation will yield a higher shoot Pb concentration. These plants are considered promising for Pb phytoremediation programs because only shoots should be harvested in Pb phytoextraction which highlights the importance of the selected species as Pb accumulators (Huang *et al,* 1997) [22].

Although Zn is essential trace element, high levels can cause harmful health effects. According to some author toxicity level of this element is around 300 µg/ g d.w. The upper toxic levels of Zn in various plants range from 100 to 500 µg/g d.w (Waganov and Nizharadze,1981) [42]. The highest Zn concentration in the root (15060 µg/g) was attained by *Phragmite australis*. The roots are thought to be important for zinc uptake (Aubert and Pinata, 1997) [5]. It was noted that the highest zinc concentrations in roots of *Phragmite australis* and *Citrullus colocynthis* were associated with high concentrations in soils at the same place. Previous studies on the accumulation of various metal ions by native plants have shown that the deposition of most metals was higher in roots than the other parts of plants (Zaranyika and Ndapwadza, 1995, Chandra and Kulshreshtha, 2004) [13, 46]. *Phragmite australis* was tested for concurrent removal of Zn. This plant has removed the metal successfully without production of toxicity.

The mean concentration in normal plants (above ground tissues) is 66 µg/g (Outridge and Noller, 1991) [35], and the toxic level is up to 230 µg/g (Borkrit *et al,*1998 and Long *et al,* 2003) [31, 38]. The ranges of Zn in plants presented here were generally higher than the levels reported for other plants (Cardwell *et al,* 2002) [11]. The results obtained by Aboulroos *et al.* (1996) [40] indicated that Zn content of plant increased with increasing levels of Zn in the soils. The research done by Kandil *et al,* (2003) [24] found highly significant correlations between the soil content of macro, micro-nutrients and heavy metals and its accumulation in roots of plants.

Nickel is used extensively as catalyst in the chemical and food industry, as prime materials for the reduction of paints and batteries, and in the electroplating industry. According to Kabata-Pendias, and Pendias (1984) [23], the normal Ni content of terrestrial plants growing in uncontaminated soils was found to be in range of 0.1- 3.7 ug/g for Ni. Our results showed that concentrations of Ni in the investigated species were higher than the normal plant, and this shows that these plants had a strong ability to tolerate this element. Heavy metal concentrations in roots of *Cannabis sativa* and *Argemone maxicana* increased in the following pattern: Cu > Cr > Ni > Cd. This may indicate that all four metals come from similar sources of contamination. Moreover, increased concentrations of four metals in roots system were due to the presence of plaque, (Sundby *et al,* 1998) [38] with high pH conditions (> 7.0) which enhanced metals uptake into roots (Weis and Weis, 2004) [44].

Bioconcentration factor

Accumulation of selected metals varied greatly among plant species and the uptake of an element by a plant is primarily dependent on the plant species, its inherent controls and the soil quality (Chunnilal *et al,* 2005). Large number of factors control metal accumulation and bioavailability associated with soil and climatic conditions, plant genotype and agronomic management, including: active/passive transfer processes, sequestration and speciation, redox states, the type of plant root system and the response of plants to elements in relation to seasonal cycles (Kabata-Pendias and Pendias, 1984) [23]. Structure of the sediment has also been considered very important that affect the extent of the metals taken up by the plants. Metal solubility in soils is predominantly controlled by pH, and oxidation state of the system (Ghosh and Singh, 2005) [20]. The results indicated that soils of study area were loam and sandy texture and were neutral in nature with pH greater than 6.6.

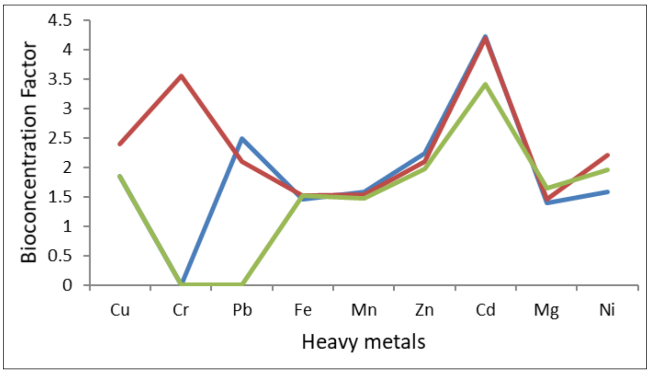

Fig 1: Graph showing comparative Bioconcentration Factor in *Cannabis sativa* during summer season at three sites

Neutral and high soil pH can stabilize soil toxic elements, resulting in decreased leaching effects of the soils toxic elements. Moreover, toxic elements may also become stabilized due to slightly basic soil pH which may result in less element concentrations in the soil solution. This may restrain the absorbability of the elements from the soil solution and translocation into plant tissues (Liu *et al,* 2005) [30]. Most of plant species under investigation had BCF >1, although the concentration of heavy metals remained below 1000 ug/g. In general, BCF values of Cd, Cu, Ni and Zn were highest as compared to other metals. Heavy metals tolerant species with high BCF can be used for phytostabilization of contaminated soils as these species retain metals in their roots and limit metal mobility from roots to shoots after absorption by roots of the plant (Cui *et al,* 2007) [17].

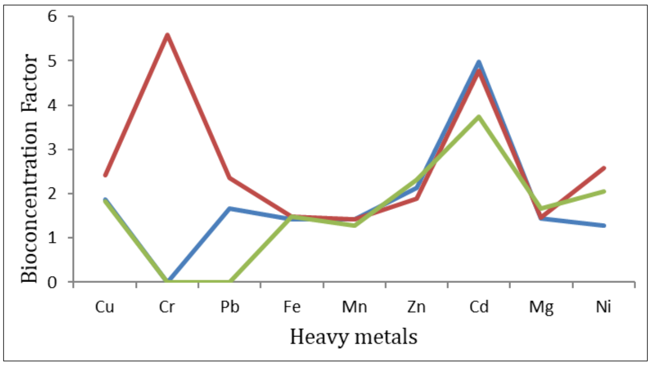

Fig 2: Graph showing comparative Bioconcentration Factor in *Argemone maxicana* during winter season at three sites

High metal accumulation may be attributed to well-developed detoxification mechanism based on sequestration of heavy metal ions in vacuoles, by binding them on appropriate ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of metallic ions (Cui *et al,* 2007) [17] and metal exclusion strategies of plant species (Ghosh and Singh, 2005) [20]. Plant species with high TF values were considered suitable for phytoextraction generally requires translocation of heavy metals in easily harvestable plant parts i.e. shoots (Yoon *et al,* 2006). According to Gosh and Singh (2005) [20, 45] phyto-extraction is a process to remove the contamination from soil without destroying soil structure and fertility. The results of the present study highlighted that both plants had relatively low BCF (1.345-1.524) for Fe in comparison to other metals. The elevated concentration of Fe in roots of plants under investigation and low translocation in above ground parts indicated their suitability for phytostabilization of this element in the study area.

Conclusion

Results indicated that *Cannabis sativa* and *Argemone maxicana* are accumulator for the studied heavy metals. The concentrations of heavy metals in soils have the sequence of "Fe > Zn >Cu >Pb > Ni > Cr > Cd" and in plants the trend was also "Fe > Zn >Cu >Pb > Ni > Cr > Cd. Roots of both the plants show high concentrations of all studied metals, except Cd, and thus are the best biomonitors for heavy metals decontamination in the studied area. The bioconcentration factor (BCF) values of Cd, Zn, Pb and Ni were highest as compared to other metals**.** These plant species can be used as hyper-accumulators and suitable for phytoextraction. However, these plants had relatively low BCF for Fe in comparison to other metals. The elevated concentration of Fe in roots of studied plants and low translocation in above ground parts indicated their suitability for phytostabilization of this element in the study area. The present study shows that these plant species can be suitable option for phytoremediation. Biotechnological and genetic engineering-based approaches can be used to enhance the naturally occurring plants to detoxify hazardous compounds.

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