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*Full Length Research Paper*

# Remediation of a heavy metal polluted soil using weedy species; *Ageratum conyzoides* L. and *Chromolaena odorata* (L.) R.M. King & H. Robinson

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**This study investigated the remediation potential of *Ageratum conyzoides* and *Chromolaena odorata.* The objectives of this research were to: (i) assess the amounts of Pb, Ni and Cr that *C. odorata* and *A. conyzoides* remove from polluted soil (ii) Determine which species best accumulated these heavy metals in their shoot. (iii) Assess the extent to which Pb, Ni and Cr affected the growth of these species. The plants species were raised from seeds in a greenhouse using a dumpsite soil containing concentration of heavy metals (Pb, Ni, Cr) above standard levels. The ability of *A. conyzoides* and *C. odorata* to grow and accumulate Pb, Ni and Cr was assessed for ten weeks. The height of these plants was measured on a weekly basis and the concentration of heavy metals was determined at the end of the study. *C. odorata* and *A. conyzoides* significantly depleted the amounts of heavy metals in the dumpsite soil. The mean concentration of Pb and Cr were reduced by 17.91% and 24.04% respectively in the dumpsite soil after the growth of *A. conyzoides* but there was no significant difference in Ni concentration. A significant decrease was observed in the concentrations of the Pb (17.39%), Ni (81.57%) and Cr (54.12%) in the dumpsite soil after the growth of *C. odorata. A. conyzoides* had a higher translocation factor for Lead (1.34) than for Chromium (0.15), but it was highest for Ni (6.34). *C. odorata* also had a low translocation factor for both Ni (0.47) and Cr (0.57) compared to Pb (5.83). There was no significant difference in the heights of these species throughout the experiment. Certain plants have the ability to tolerate heavy metal soil contamination without their growths being significantly impaired as was the case with *C. odorata* and *A. conyzoides* in this study. *C. odorata* has a higher capability to remove heavy metals from soil than *A. conyzoides.* Both species are potential candidates for the bioremediation of Pb and Ni in soils.**

**Keywords:** Ageratum conyzoides, Chromolaena odorata, heavy metals, weedy species, phytoremediation.

## INTRODUCTION

Auto-mechanic activities are one of the major sources of heavy metal entry into the environment in Nigeria (Adewole and Uchegbu, 2010) among others which include disposal of degraded sediments, fertilizer and pesticide application (Aguilar *et al.,* 2013) to mention a few. Auto-mechanic workshops are found in clusters of open plots of land in the vicinity of urban towns and cities (Nwachukwu *et al.,* 2010; Nwachukwu *et al.,* 2011). Within the clusters are artisans who specialize in auto-electrical repairs, automatic or standard transmission engine trouble-shooters, auto-body spray-painters, battery chargers, welders etc. Each of these activities generate various types of waste including gasoline, diesel, spent engine oil, paint and both metal and plastic scraps which are disposed of by dumping in nearby fields and surrounding areas. The most frequently encountered heavy metals in auto mechanic workshop soils and associated wastes include Copper, Lead, Nickel,

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Chromium, Cadmium, Zinc, Manganese and Nickel, all of which pose risks to human health and the environment (Oguntimehin and Kolad, 2008, Pazand *et al.,* 2018). The polluting effects of mechanic site activities in Nigeria have received limited attention even though these activities have been shown to produce harmful wastes (Udebuani *et al.,* 2010) which are improperly handled by artisans operating auto-mechanic workshops in Nigeria (Omofonmwan and Osa-Edoh, 2008) leading to large scale environmental degradation (Mwambazambi, 2010). Therefore, there is need to observe the effects of the heavy metals encountered in auto-mechanic wastes on the growth of plants and to investigate plants' potential to remediate the harmful effects of heavy metal accumulation on the soil through bioaccumulation as a cost effective alternative.

Heavy metals that are available for plant uptake are those that are present as soluble components in the soil solution or those that are easily solubilized by root exudates (Blaylock 2000) and they are taken up by a process referred to as rhizofiltration (Prasad and Freitas, 2005). Although plants require certain heavy metals for healthy growth, excessive amounts of these metals can become toxic. The ability of plants to accumulate essential metals equally enables them to acquire other non-essential metals (Djingova *et al.,* 2000). As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant directly and indirectly. Some of the direct toxic effects caused by high metal concentration include inhibition of cytoplasmic enzymes and damage to cell structures due to oxidative stress (Assche *et al., 1990*) (Jadia *et al., 2009*). An example of indirect toxic effect is the replacement of essential nutrients at cation exchange sites of plants (Taiz *et al., 2002*). Furthermore, the negative influence heavy metals have on the growth and activities of soil microorganisms may also indirectly affect the growth of plants. For instance, a reduction in the number of beneficial soil microorganisms due to high metal concentration may lead to decrease in organic matter decomposition leading to a decline in soil nutrients. Enzyme activities useful for plant metabolism may also be hampered due to heavy metal interference with activities of soil microorganisms. These toxic effects (both direct and indirect) lead to a decline in plant growth (Bhattacharyya *et al.,* 2008) which sometimes results in the death of the plant (Schaller *et al.,*1991).

The effect of heavy metal toxicity on the growth of plants varies according to the particular heavy metal involved in the process. For metals such as Lead, Cadmium, Mercury, and Arsenic which do not play any beneficial role in plant growth; adverse effects have been recorded at very low concentrations in the soil. Kibra (2008) recorded significant reduction in height of rice grown on a soil contaminated with 1 mgHg/Kg. Reduced tiller and panicle formation also occurred at this concentration in the soil. For Cd, reduction in shoot and root growth in wheat occurred when soil concentration was as low as 5 mg/L (Ahmad et al*.,* 2012). Most of the reductions in growth parameters of plants growing on polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activities of some enzymes (Kabata-Pendias, 2001). Over 400 hyperaccumulator plants have been reported including members of the Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae. The Brassicaceae is a very important group when heavy metal accumulation is concerned, with several species being able to hyperaccumulate more than one metal (Prasad and Freitas*,* 2003). This is also applicable to many species in Asteraceae as demonstrated by previous works involving *Tithonia diversifolia* and *Helianthus anus* (Adesodun *et al*., 2010), *Chromolaena odorata* (Harrison, 2011) and *Tithonia diversifolia* with *Ageratum conyzoides* (Dada *et al.,*2012)*.*

## MATERIALS AND METHODS

### Experimental protocol

The substrate for this experiment was heavy metal contaminated soil for the treatment pots and uncontaminated or normal soil for the control pots. Samples of suspected heavy metal polluted soil were initially collected from a dump site at a Mechanic Village (7º 25' 49"N, 3º 54' 33"E). Two sample points were randomly located for collections. Separate collections were analyzed to quantify the amounts of heavy metals, namely Lead (Pb), Chromium (Cr) and Nickel (Ni). The dumpsite soil contained heavy metals above standard levels and comprised metal scraps from vehicle parts, leaf litter and some house-hold garbage like used clothes, polythene bags, tins etc. About 90kg of soil was excavated to and transported to the nursery of the Department of Botany, University of Ibadan.The samples were air dried for 7 days. About 60 kg of uncontaminated top soil was also collected according to the method of Adenipekun *et al.,*(2013).The collected soil samples were then sieved with a 1mm wire mesh sieve and 5 Kg of soil was potted in buckets perforated at the base. Mature seeds of *Ageratum conyzoides* and *Chromolaena odorata* were collected from the University of Ibadan.

### Cultivation and plant maintenance

Planting was carried out in the nursery of the Department of Botany, University of Ibadan. According to the method of Garcia *et al.,* (2004) the seeds were planted in triplicate in the polluted soil as well as the unpolluted soil as control. The experimental design for this experiment was a Completely Randomized Design. About 15-20 cleaned seeds of the two plant species were broadcast unto the labeled pots containing the treatment and control

soil samples respectively and then irrigated to field capacity. Seedlings in each pot were thinned to three plants per pots on the fourth week of planting.
The experiment was terminated after ten (10) weeks of planting and the plants were harvested. Soil samples from the plant rhizosphere were collected in nylon bags. The roots of the plants were rinsed thoroughly in water before been separated from the shoot.

**Laboratory analysis**

Plant heavy metal (Chromium, Cr; Nickel, Ni and Lead, Pb) were assessed using the method of Odu *et al.,* (1968). Plant tissue sample was finely ground after drying at approximately 80ºC for 48 hours. Dried plant tissue (1g) was then weighed into a 100ml Berzelius beaker and 5ml $HNO_3$ and 2ml $HCLO_4$ was added (10-15mls) before it was digested with heat to a final volume of 3 to 5ml. Water was later added and the digest was filtered through an acid washed filter paper into a 25 ml volumetric flask. 5ml of digest was diluted to the 25 mlmark with distilled water and a spectrophotometer was used to determine the concentration of the heavy metals of interest. (Buck 210 AAS, 2005, USA)
Soil sample digestions and analysis were done as described by Ho and Tai*,* (1998). Three selected heavy metals (Chromium, Cr; Nickel, Ni and Lead, Pb) were assayed. Air-dried samples were ground using mortar and pestle, and sieved with 2mm mesh size. Two grams (2g) of each of the soil samples was accurately weighed and treated with 10 ml aliquots of high purity concentrated $HNO_3$. The mixture was heated on a hot plate until the sample was almost dry and then cooled. This procedure was repeated with another 10ml of concentrated $HNO_3$ followed by 10 ml of 2M HCl to re-dissolve the residue. The extracts were then filtered through Whatman filter paper (no. 42) into 50ml capacity bottle and brought up to volume with doubled distilled water.
Heavy metal concentrations were determined using Atomic Absorption Spectrophotometer (Buck 210 AAS, 2005, USA).
The translocation factor (TF) of heavy metals from roots to shoots (Zu *et al.,* 2005) was calculated as follows:
$TF = C_s / C_r$
Where, TF is Translocation Factor $C_s$ represents metal's concentration in plant shoots and $C_r$ represents metal's concentration in plant roots.

**Data analysis**

The concentration of Chromium, Cr; Nickel, Ni and Lead, Pb were compared between the polluted soil and the rhizosphere of the two plant species using a paired*t*-test. The concentration of the three heavy metals in the roots and shoots of *Ageratum conyzoides* and *Chromolaena odorata* were assessed using an independent *t*-test.The difference in plant heights for each species grown in dumpsite and control soil was also assessed using independent *t*-test.

**RESULTS**

The results of the physicochemical characteristics of the soils investigated are summarized in Table 1a, 1b and 1c. It was observed that the polluted soil was higher in Lead, Chromium and Nickel concentrations compared with the standard safe concentrations as well as the total nitrogen content, pH, exchangeable base and micronutrient content in comparison to the unpolluted soil. However, the unpolluted soil had a greater proportion of clay in its particle composition than the polluted soil. (Tables 1a, b, c).
The ratio of metal accumulation in the shoot and root of *A. conyzoides* and *C. odorata* are expressed in Table 2
*A. conyzoides* had a higher translocation factor for Lead than for Chromium. However, this ratio was highest for Nickel. Moreover, *C. odorata* had very low translocation factor ratios for Ni and Cr unlike its ratio for Lead.
Figures 1a and 1b show the difference in metal concentration in the polluted soil before and after the experiment for *A. conyzoides* and *C. odorata* respectively.
There was a significant difference in the amounts of Pb and Cr in the polluted soil after growth of *A. conyzoides* but no significant difference in the concentration of Ni. However, a significant difference was observed in the concentrations of the three heavy metals in the dumpsite soil after the growth of *C. odorata.*
Fig. 2a and 2b show the difference in metal accumulation by *A. conyzoides* and *C. odorata* in the roots and shoots respectively.
*C. odorata* accumulated a higher concentration of Ni and Cr in its roots than *A. conyzoides.* However, more lead was accumulated by the roots of *A. conyzoides.* Moreover, *C. odorata* accumulated more Pb and Cr in its shoot than *A. conyzoides. A. conyzoides* only accumulated more Ni in its shoot than *C. odorata* Fig 3a and 3b show the variation in height of *A. conyzoides* and *C. odorata* respectively grown in the dumpsite and control soil for seven (7) weeks.
There was no significant difference in the growth *A. conyzoides* and *C. odorata* in the dumpsite soil and control soil.

**DISCUSSION**

Soils from dumpsites have been reported to have higher nutrient concentration than control soils (Obianefo *et al.,* 2017) and this could be due to the higher influx of materials into soils at dumpsites and higher rate of decomposition. The solubility of nutrients in the soil has been reported to be correlated to pH (Obianefo *et al.,* 2017). The higher pH in the dumpsite soil has also been

**Table 1a**: Soil physiochemical characteristics.

| S/N | I.D | pH | T.O.C (g/kg) | T.N (g/kg) | Av. P(mg/kg) | Exch. Acid $H^+$ | Silt (g/kg) | Clay (g/kg) | Sand (g/kg) |
|---|---|---|---|---|---|---|---|---|---|
| 1 | C | 6.4 | 14.04 | 1.99 | 30.04 | 1.2 | 114.0 | 94.0 | 792.0 |
| 2 | T | 7.3 | 50.13 | 2.96 | 67.78 | 1.6 | 114.0 | 84.0 | 802.0 |

Key: C=control (unpolluted soil), T=treatment (polluted soil), T.O.C=total organic carbon, T.N=total nitrogen, Av. P=average phosphorus.

**Table 1b**: Soil micro and macro elements.

| | | (Cmol/kg) | | | | | (mg/kg) | | |
|---|---|---|---|---|---|---|---|---|---|
| S/N | I.D | Ca | Mg | K | Na | Mn | Fe | Cu | Zn |
| 1 | C | 6.29 | 1.37 | 0.40 | 0.52 | 101.10 | 134.00 | 11.50 | 16.63 |
| 2 | T | 45.31 | 3.79 | 0.58 | 0.57 | 70.30 | 158.00 | 23.90 | 230.50 |

Key: C=control (unpolluted soil), T=treatment (polluted soil), T.O.C=total organic carbon, T.N=total nitrogen, Av. P=average phosphorus.

**Table 1c**: Heavy metal concentration in initial test samples (X±SD).

| | Pb (mg/kg) | Cr (mg/kg) | Ni (mg/kg) | pH |
|---|---|---|---|---|
| Polluted soil | 367.00 ± 83.43 | 194.73 ± 6.47 | 69.28 ± 4.07 | 6.85 ± 0.07 |
| Unpolluted soil | 30.90 ± 0.00 | 13.10 ± 0.00 | 14.40 ± 0.00 | 6.1 ± 0.00 |
| Standard | <300.00 | <64.00 | 35 | --- |
| Source | WHO(1996) | CCME(1991) | WHO(1996) | |

Key: C=control (unpolluted soil), T=treatment (polluted soil), T.O.C=total organic carbon, T.N=total nitrogen, Av. P=average phosphorus.

**Table 2:** Translocation factor of Pb, Ni and Cr in *A. conyzoides* and *C. odorata.*

| | Pb | Ni | Cr |
|---|---|---|---|
| *A. conyzoides* | 1.34 | 6.34 | 0.15 |
| *C. odorata* | 5.83 | 0.46 | 0.57 |

attributed to microbial activity and liming materials in the solid waste (Ideriah *et al.,* 2006).

Though the mechanisms by which plant hyperaccumulate heavy metal is not yet fully understood, tissue-specific expression of proteins, high metal-chelator concentations (Viehweger, 2014) and genetic over-expression of particular plants encoding transmembrane-transporters has been shown to drive the uptake, translocation and sequestration of heavy metals in cellular components of such plants especially in the shoot. This mechanism is believed to be a viable plant defence when acting in concert with organic defensive compounds to discourage attacks from their natural enemies and there have been evidences supporting this "elemental defence" hypothesis in plants (Rascio and Navari-Izzo, 2011).

One way plant roots of *A. conyzoides* and *C. odorata* were able to solubilize and absorb appreciable amounts of Pb, Ni and Cr from the dumpsite soil was by acidifying their soil environment with protons extruded from the roots. A lower pH solubilizes metal precipitates and releases soil-bound metal ions into the soil. A similar mechanism has been observed for Fe mobilization in

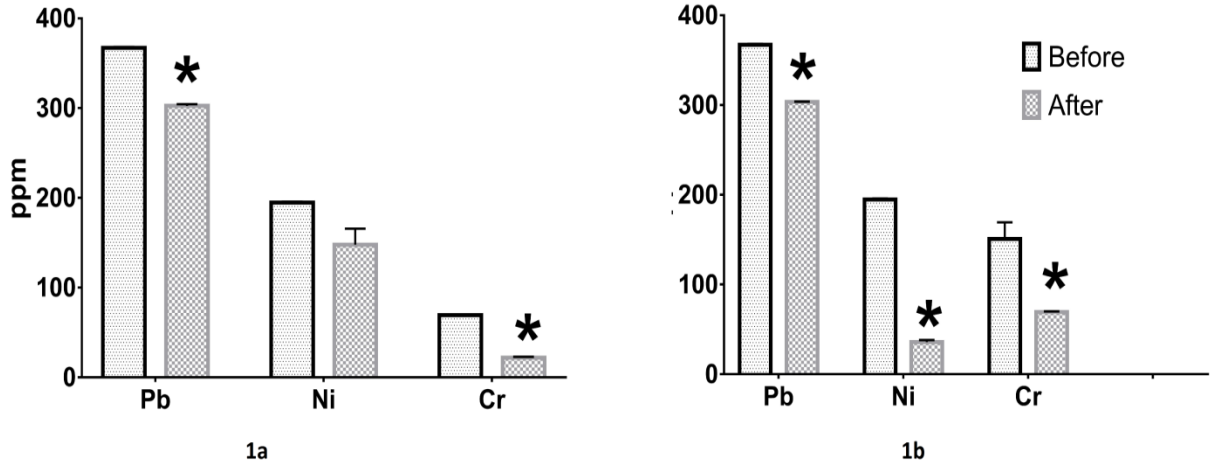


**Fig. 1**: Concentration of heavy metals in dumpsite soil before and after the growth of *A. conyzoides* and *C. odorata* respectively. Asterisks denote significant differences.

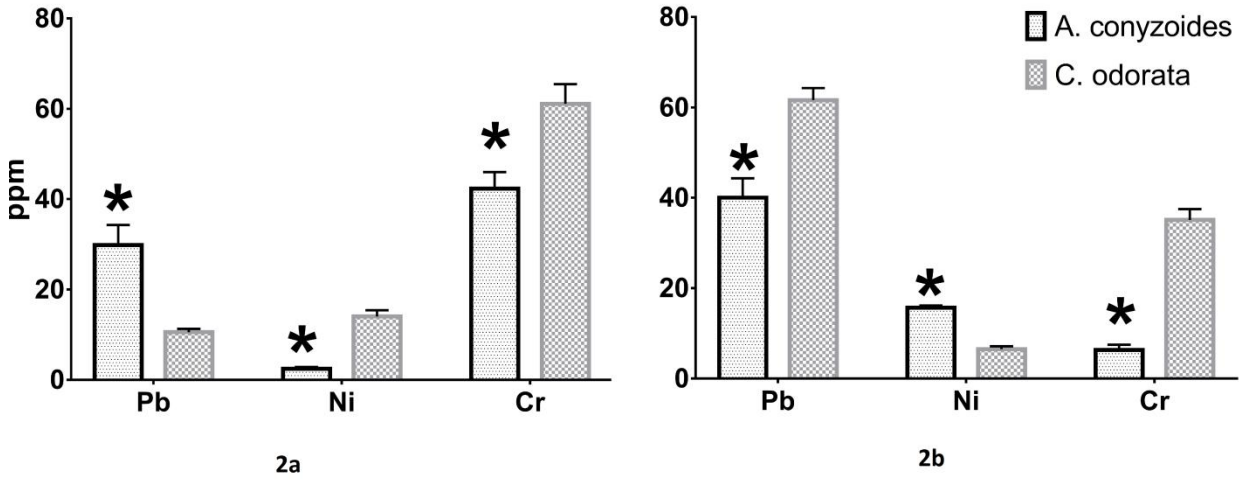


**Fig. 2:**Metal accumulation by *A. conyzoides* and *C. odorata* in the roots and shoots respectively. Asterisks denote significant differences.

some Fe-deficient dicotyledonous plants (Crowley et al*.,* 1991). The initial pH in the dumpsite soil was recorded at 6.9 but at the end of the experiment, it was observed to have increased to 7.3. The release of H+ and OH- ions in the rhizosphere is associated with maintaining cation and anion balance in the plant during the ion uptake process. When more cations are absorbed, H+ ions are released and pH decreases. (Mengel et al*., 2001*).

Agunbiade et al*.,* (2009) reported the ability of *Chromolaena odorata* to accumulate and serve as biomarker to Lead. *C. odorata* can be described as having remediated Pb in the dumpsite soil with a translocation factor for Pb of 5.83, greater than one as explained by Salido (Salido *et al.,* 2003). The phytoremediation of the Nickel polluted soil was however most appreciable by *A. conyzoides* with a translocation factor of 6.34. *A. conyzoides* and *C. odorata* had very low translocation factor for Cr (0.15 and 0.57 respectively) and therefore showed very low remediation prospects for Cr. Many studies have demonstrated that chromium uptake from soils or nutrient solution and translocation to plant cells is very low (Patterson *1971*).

*Chromolaena odorata* showed no significant difference in height between the treatment and control pots at 5% level

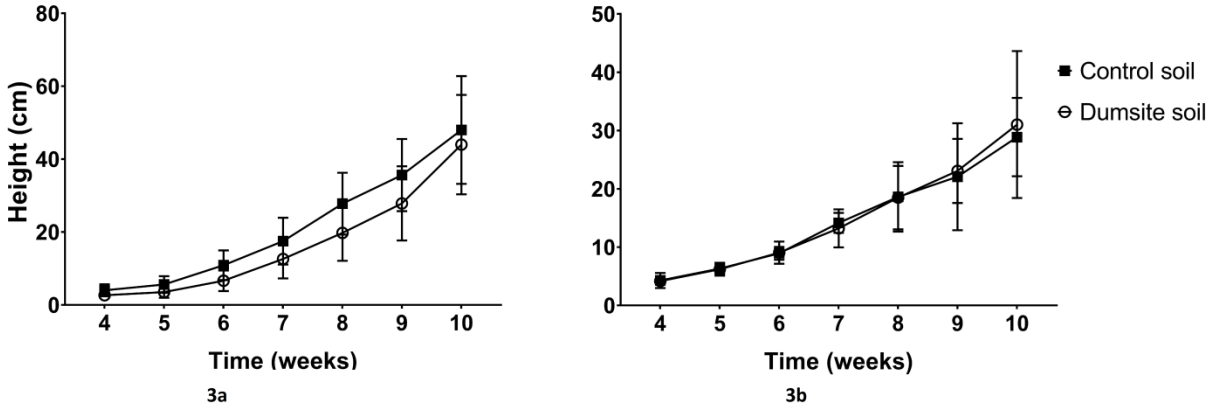


**Fig 3:** Height of *A. conyzoides* and *C. odorata* respectively. Error bars: Standard errors.

of significance through the fourth and ninth weeks. This data indicates that the growth of the plant under the set experimental conditions was not adversely affected by the heavy metal pollution in the treatment pots. This result was in accordance with the work of Harrison, (2011) where he reported that *C. odorata* (L) has the capability of thriving in heavy metal contaminated soils. Plant metal hyperaccumulation is associated with metal hypertolerance revealing another strategy of detoxification where such hyperaccumulating plants are able to grow inspite of high heavy metal soil concentration (Viehweger, 2014).

## CONCLUSION

This study addressed three questions being (i) the occurrence and extent of reductions in the amounts of Pb, Ni and Cr in the soil after the growth of *C. odorata* and *A. conyzoides*. (ii) The identification of a species which best accumulated Pb, Ni and Cr in the shoots than root (iii) the effects of Pb, Ni and Cr on the growth of both species.

*C. odorata* has a higher capability to deplete soil Pb, Ni and Cr levels than *A. conyzoides.* These plants were able to alter soil pH to increase the bioavailability of nutrients and heavy metals. *A. conyzoides* is best able to remediate Ni polluted soils while *C. odorata* is best applicable in Pb polluted soils. None of the plants were able to remediate Cr in the dumpsite soil. Neither plant species had their growth significantly affected by heavy metal concentrations at the experimental levels.

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