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Source and Health Implications of High Toxic Metal Concentrations in Illicit Tobacco Products

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A significant flux of heavy metals, among other toxins, reaches the lungs through smoking. Consequently, contaminated soil is usually avoided for tobacco cultivation. Here we compare the heavy metal concentrations in tobacco from a sample of 47 counterfeit products, representative of the substantial market for these products in the U.K., with their genuine equivalents and find significantly higher concentrations of heavy metals in the counterfeits. Trace element patterns suggest that over-application of fertilizers (phosphate and/or nitrate) is the most likely cause. Nitrogen isotopes showed no significant enrichment in ^{15}N (δ^{15}N range from +1.1 to +4.6‰ in counterfeits and from +2.5 to +3.3‰ in genuine tobaccos) as might be expected from a sewage or manure source of nitrate, and a mineral phosphate source is considered the more likely source of metals. Stable carbon isotopes in the same tobaccos have a wide range (δ^{13}C −18.3 to −26.4‰), indicating the influence of multiple controls during cultivation and possibly post-harvesting. A review of the health effects of heavy metal transfer from tobacco via smoke to the lungs indicates that habitual smokers of counterfeits may be risking additional harm from high levels of cadmium and possibly other metals.

Introduction

Smoking delivers heavy metals (the term is used sensu lato to include some lighter metals and metalloids; *1*) to the lungs (*2*), particularly the more volatile metals such as cadmium and mercury that partition preferentially into the smoke phase on combustion (*3*−*6*). Some of these readily pass into the bloodstream and may accumulate in specific organs (*7*, *8*). Indeed smoking has long been considered a major source of several heavy metals in blood and various organs (*2*), and cadmium in particular is regarded as one of the "strong carcinogens" in tobacco smoke (*9*) with Cd, Ni, and As currently classified "carcinogenic to humans" by the International Agency for Research on Cancer (IARC) among 87 mainly organic carcinogens. Although there are global and brand variations in the heavy metal compositions of commercial tobacco products (*5*, *6*, *10*−*14*), only a few studies have tried to link smoking-related diseases mechanistically to heavy metals derived from tobacco combustion (*15*), and there is evidence that heavy metal concentrations in commercial tobacco products consumed by the public have declined with time (*14*). Notwithstanding, it has been known for a few decades that tobacco combustion has the potential to deliver dangerous quantities of heavy metals to the lungs if the tobacco being combusted has high initial concentrations (*6*), some of which is then transferred to other organs of the body (*16*). In this regard it is noteworthy that the tobacco plant *Nicotiana tabacum* is well-known for its capacity to concentrate heavy metals from its growing environment (*17*−*19*), in particular as an accumulator of Cd in the leaf (*20*).

Any investigation of the harmful effects of commercial tobacco products must take into account potential differences between genuine bona fide brands and the illicit market, especially in countries where the latter has a significant market share. For instance, in 2000−2001 illicit tobacco was estimated to account for 25% of all cigarette sales in the United Kingdom (*21*), with genuine products legally exported but illicitly re-entering the country (contraband) accounting for about 20% and counterfeits accounting for the remaining 5%. At that time the top-selling brand had a market share of about 11%, and only four legitimate brands had a greater share of the U.K. market than counterfeits. There is evidence that counterfeit sales have grown both proportionately and in absolute terms and may now exceed the best-selling brands. Given their substantial market share and the fact that they evade all statutory controls, it is in the interests of public health to determine whether these counterfeits have any characteristics that render them more harmful than their genuine equivalents.

Here we examine the heavy metal concentrations in tobacco from a sample of 47 counterfeit cigarettes, representative of those distributed in the U.K. between late 2002 and early 2004. We compare these concentrations with their genuine equivalents and report much higher concentrations of heavy metals in this substantial subset of the nation's commercial supply. We identify potential harmful consequences to human health from these heavy metals and conclude that the typical counterfeit product adds significantly to the risks normally associated with smoking cigarettes.

The consistency of heavy metal enrichment in the illicitly manufactured product is surprising. It has not been possible to discover their sources and thus constrain the nature of their growing environments as well as industrial pollution, fertilizer applications, and other processes that could contribute heavy metals. We have, therefore, attempted to deduce any patterns in the plant growing environment from trace element abundances and the stable isotopes of nitrogen and carbon (δ^{15}N & δ^{13}C). We find strong evidence that heavy application of fertilizers is primarily responsible rather than fortuitous cultivation on geologically or industrially contaminated land.

Materials and Methods

Sample Material and Reference Standards. Samples of counterfeit cigarettes were supplied for analytical purposes by U.K. HM Customs & Excise in the form of one or two packs of 20 cigarettes for eight purported brands among the best-selling brands currently available in the U.K. Some 47 samples of counterfeits were obtained between September 2002 and January 2004. Genuine samples of the same brands were obtained simultaneously from reliable retailers for the purpose of comparison of the heavy metal concentrations.

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The eight genuine brands are not disclosed but are represented by the letters A–H.

Reference standards for tobacco are few. The best known are the Kentucky University standard cigarettes, which are designed to represent typical U.S. blends for the purpose of testing nicotine, carbon monoxide, tar, etc. However they are not intended for trace element studies and no published data were located, but we have analyzed two (KU-1R4F and KU-1R5F) as they are widely available and used in other studies of toxic properties. For trace elements, the most comprehensively analyzed tobacco standards are the Polish Certified Reference Materials CTA-OTL-1 and CTA-VTL-2 (Oriental and Virginia tobacco leaves, respectively). As well as providing certified values for many heavy metals these CTA standards are important as they specifically represent tobacco leaf whereas the KU standards represent typical U.S. cigarette blends, which include leaf and other components of the tobacco plant.

Sample Preparation. The CTA standards were supplied as dry powders. For the analysis of cigarette tobacco, we separate the tobacco component from its filter and wrapping paper and dry it in a sterilized glass beaker for 72 h at 80 °C. The dried samples are then ground in a IKA20 Mill using a WC blade for 90 s. Approximately 6 g of tobacco powder is weighed and pressed at 15 tons for 2 min in a die to produce a 32 mm diameter pressed powder pellet. Robust disks are formed without requiring the addition of a binder.

Polarized Energy Dispersive X-ray Fluorescence (EDPXRF). The analysis of plant material by XRF normally suffers from high backgrounds attributable to both primary radiation due to the X-ray tube and secondary scattered radiation from the sample (*22*, *23*). This may be overcome by the use of linearly polarized X-rays, and this approach has been applied successfully to the analysis of nonorganic elements in foliage (*24*). The instrument used in this study was a Spectro XLAB EDPXRF spectrometer equipped with a Rh anode X-ray tube with an excitation potential of up to 3.0 kW. Some 26 elements are capable of routine analysis in tobacco using this method (*24*), but only Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Sn, and Pb are considered in this study.

Stable Isotope Analysis. Approximately 1.5–2 mg of each tobacco sample is accurately weighed into a 3.5 × 5 mm tin capsule and loaded into a pneumatic autosampler. Stable isotope measurements of δ^{15}N and δ^{13}C are made using continuous flow isotope ratio mass spectrometry (CF-IRMS) using a Thermo Finnigan Delta Plus XP mass spectrometer interfaced with a Costech ECS 4010 elemental analyzer. Samples are flash-combusted, the resulting N_2 and CO_2 are then purified and carried to the mass spectrometer in a stream of high-purity helium gas. All stable isotope values are reported in per mil (‰) using the δ notation:

$$\delta^{\text{heavy}}\text{element}(‰) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}}\right) - 1\right] \times 1000$$

where R is the ratio of heavy to light isotope (^{13}C/^{12}C or ^{15}N/^{14}N) in the sample or international standard (V-PDB for δ^{13}C and AIR for δ^{15}N). Raw data are normalized using an internal standard gelatin. The method has a routine precision better than ±0.15 for both elements.

Results and Discussion

Heavy Metal Concentrations and Sources. The concentrations of 10 representative "heavy metals" in 47 counterfeit samples are presented in Table 1 along with the concentrations obtained for contemporaneous genuine samples of the same brand. The data for genuine samples are supplemented from a larger (unpublished) database obtained under precisely the same conditions. The combined data are displayed as histograms (Figure 1) where the overall trends are immediately obvious. Genuine brands have very limited variance and have modes that invariably occur at or toward the low end of the more widely dispersed distributions for counterfeits. In the cases of As, Cd, and Pb there is very little overlap, and the counterfeits have markedly higher concentrations of these elements. This is particularly significant as all three elements are known to be carried to varying degrees by the smoke phase released on tobacco combustion (*3*, *6*, *25*, *26*). Also, all three elements are listed by the International Association for Research in Cancer (IARC) as carcinogens; Cd and As as *known* human carcinogens (type 1) and Pb as a *possible* human carcinogen (type 2B) (*27*).

The pattern of heavy metal enrichment in counterfeits is reinforced by plots comparing the concentrations of each element to their concentrations in the genuine brand that they purport to represent (Figure 2). Even taking into account analytical errors Cd and Pb are clearly significantly richer in almost all counterfeits, but this is less certain for As. Fe and Zn also show significant enrichment in counterfeits and both elements enter the smoke phase, primarily into sidestream smoke (*3*, *28*). The significance of this enrichment of heavy metals in counterfeits is underlined by the fact that the mean values obtained exceed all values in a global compilation of cigarette tobaccos for the elements As, Cd, Cu, Pb, and Zn (Table 3 in Jung et al.; *5*).

It is inconceivable that counterfeiters deliberately and consistently add heavy metals to their products; there is no obvious reason to do so. Heavy metals in tobacco are primarily derived from the soil in the cultivation environment, and the plant is well-known as an effective sequestrator of heavy metals (*29*).

The principal anthropogenic sources of heavy metal contamination of soils have been identified (*30*), being

1. metalliferous mining, smelting, and metallurgical industries
2. agricultural and horticultural materials
3. sewage sludges
4. fossil fuel combustion
5. electronics, especially disposal
6. chemical and other manufacturing industries
7. waste disposal
8. sports shooting and fishing
9. warfare and military training

Sources 1 and 4–9 can, on a regional scale, be regarded as localized. As we understand that these counterfeits probably derive from different regions of the world, the consistency of heavy metal enrichment coupled with the scale of production effectively rules out such localized sources. Only the regional-scale application of agricultural materials (fertilizers) and sewage sludge/manure (commonly used as nitrate fertilizers) are likely provide the observed consistency of contamination.

Another factor influencing tobacco available for the counterfeit market is economics. Growing tobacco on strongly acidic but unpolluted soils is known to enrich the lower leaves in cadmium by as much as 5-fold (*31*). After this became known, the major manufacturers of genuine brands tended to avoid these crops and sought a supply of tobacco cultivated on less acid soils. A possible consequence is that the surplus crop found its way into counterfeit production. As a major factor in the current U.K. heavy metal enrichment of counterfeits, this is unlikely to be significant as all the analyzed heavy metals are more or less enriched (Figure 1), including those such as Cr that are not influenced by soil acidity.

Both mineral phosphate and organic nitrate fertilizers are known as potential sources of several heavy metals such that their application to soils is often regulated in developed countries (*30*). Phosphatic and sewage-based fertilizers vary considerably in their heavy metal contents (*29*), but average

TABLE 1. Concentrations of Metals and As Analyzed in 47 Counterfeit Samples Seized in the U.K. during 2003 and Early 2004 and Their Genuine Contemporary Equivalents[a]

		Mn (mg/kg)		Ni (mg/kg)		Cu (mg/kg)		Zn (mg/kg)		Cd (mg/kg)		Cr (mg/kg)		Fe (mg/kg)		As (mg/kg)		Sn (mg/kg)		Pb (mg/kg)	
no.	brand	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.	count.	gen.
1	A	277		0.9		16.0		46		4.9		1.2		434		1.5		0.5		8.4	
2	A	272		1.2		14.3		54		3.7		3.1		548		1.3		0.6		7.4	
3	A	305		2.0		12.2		59		3.8		3.2		428		0.5		0.4		3.7	
4	A	121	158	1.4	1.9	14.1	12.8	29	34	1.7	0.5	2.3	2.5	566	323	1.0	0.3	0.6	0.5	1.8	0.8
5	A	228		2.3		10.2		40		4.7		2.9		510		1.4		1.0		5.8	
6	A	173		2.4		15.4		48		2.7		3.9		601		0.0		0.0		5.0	
7	A	320		2.5		22.1		87		3.7		0.0		469		1.2		0.0		6.3	
8	A	309		3.3		42.2		100		4.9		3.1		306		0.8		1.1		7.7	
9	B	255		1.4		15.8		50		4.4		1.7		436		1.2		0.7		7.6	
10	B	247		2.3		16.3		51		4.2		2.0		498		1.4		0.6		7.4	
11	B	188		1.5		20.8		61		2.8		1.9		347		0.6		0.2		3.3	
12	B	146		2.6		19.6		33		1.1		3.5		669		1.3		0.7		2.0	
13	B	154	169	2.6	1.3	16.9	12.1	33	32	1.6	0.5	3.3	2.0	854	293	1.2	0.0	0.3	0.4	1.9	0.6
14	B	232		2.9		15.6		51		2.4		3.3		455		1.2		0.7		2.1	
15	B	236		2.8		11.8		35		2.2		2.8		505		0.6		0.5		2.9	
16	B	316		3.4		20.4		73		3.8		5.1		430		1.0		1.0		2.7	
17	B	258		2.5		13.8		44		3.9		3.9		677		0.7		2.1		10.3	
18	C	263		6.4		13.2		36		1.7		5.5		876		0.2		0.2		1.3	
19	C	61		3.1		26.5		26		0.5		4.9		676		0.6		0.3		4.0	
20	C	55	162	2.3	1.8	26.1	11.7	28	29	0.2	0.5	3.9	2.0	568	340	0.6	0.3	0.2	0.4	2.0	0.6
21	C	260		2.6		17.9		85		6.0		3.9		452		1.3		1.0		7.6	
22	C	234		1.3		14.1		48		5.6		2.8		399		1.5		0.8		5.8	
23	D	283	151	1.2	1.1	12.6	11.7	59	26	2.9	0.6	2.1	1.3	720	264	0.7	0.2	0.4	0.3	5.2	0.4
24	E	265		1.7		18.9		53		3.1		2.9		592		0.8		0.3		5.8	
25	E	540	174	2.3	2.7	14.0	16.2	59	40	6.1	0.8	3.3	2.8	1444	576	2.1	0.7	0.6	0.5	8.2	0.5
26	E	226		9.2		16.4		39		2.0		7.7		966		0.6		0.8		0.6	
27	E	308		3.1		9.1		43		3.2		5.0		829		1.6		0.3		2.7	
28	F	195		2.7		16.6		44		3.6		3.0		530		1.1		0.4		5.0	
29	F	247	157	2.4	2.0	18.2	12.3	51	30	3.6	0.8	4.1	2.1	538	350	0.8	0.0	1.6	0.3	3.1	0.9
30	F	269		1.6		15.8		57		5.0		3.5		583		1.8		0.7		6.7	
31	G	214	172	3.3	2.0	19.1	14.9	38	35	1.1	0.5	3.9	3.1	810	343	0.4	0.0	0.5	0.4	1.4	0.8
32	H	157		4.2		17.6		46		1.1		4.5		721		0.6		0.2		1.0	
33	H	128		5.8		16.6		45		0.4		3.8		504		0.5		0.6		0.3	
34	H	217		2.1		19.5		51		2.9		2.9		555		0.6		0.8		4.9	
35	H	154		4.1		16.1		33		1.6		4.8		845		0.6		0.5		0.6	
36	H	106		3.7		17.3		37		1.0		3.2		361		0.6		0.5		0.8	
37	H	107		3.3		16.3		35		0.8		2.6		329		0.6		0.3		0.7	
38	H	141		5.6		17.7		51		0.4		5.6		604		0.6		0.3		0.0	
39	H	273	167	2.6	2.0	12.9	12.3	46	31	3.0	0.6	3.8	2.2	580	336	0.9	0.4	0.4	0.5	2.4	0.9
40	H	301		2.9		12.9		48		3.0		3.8		532		0.0		0.3		3.2	
41	H	210		2.5		14.6		43		4.1		2.8		490		1.2		0.7		5.4	
42	H	229		2.2		14.8		58		2.4		3.5		443		0.4		0.6		3.5	
43	H	128		2.4		17.7		29		1.5		4.8		592		0.6		0.7		4.7	
44	H	212		2.4		13.3		50		3.5		4.6		732		0.8		0.7		6.2	
45	H	230		1.9		11.7		45		4.5		3.8		569		1.7		1.1		3.7	
46	H	253		2.0		14.5		54		5.5		4.6		702		0.8		0.8		6.7	
47	H	328		1.7		15.3		70		4.2		3.0		380		0.8		0.8		4.4	
mean		226	164	2.8	1.8	16.7	13.0	49	32	3.0	0.6	3.5	2.3	588	353	0.9	0.2	0.6	0.4	4.1	0.7
median		232	164	2.5	1.9	16.0	12.3	48	31	3.0	0.6	3.5	2.2	555	338	0.8	0.3	0.6	0.4	3.7	0.7
SD		84	8	1.5	0.5	5.2	1.7	15	4	1.6	0.1	1.3	0.6	200	95	0.5	0.2	0.4	0.1	2.6	0.2

[a] Brands labeled A–H. Means and standard deviations for the whole data set included to indicate magnitude of the heavy metal enrihcment across the sector. On average Cd and Pb are enriched by about half an order of magnitude.

concentrations can assist in gauging potential effects (*30*). Bioavailability factors are also highly variable among the heavy metals (*29*); consequently, neither concentrations in the soil nor concentrations in the fertilizer necessarily control the ultimate concentration in the plant (*32*). Conversely, even low soil and fertilizer concentrations of elements such as cadmium that typically have high bioavailability can lead to toxic levels in those plants that accumulate those elements, although soil pH, redox potential, clay content, and other factors also influence phytoavailability (*32*). Notwithstanding, the tobacco plant is known to accumulate some heavy metals, especially cadmium (*17*, *19*), and leaf from tobacco grown in Maryland on a soil–sludge mixture rich in heavy metals has been shown to be enriched in Zn, Cu, Mn, Ni, and Cd (*33*). Tobacco grown in an experiment using extreme application rates of sludge emitted significantly higher Cd in the particulates of mainstream smoke than a control sample grown on the same soil without sludge (*34*).

Phosphate and Nitrate Fertilizers. If heavy metal enrichment in counterfeits is attributable solely or largely to one or other of the fertilizers implicated above, then a combination of the heavy metals concentrations in typical fertilizers and knowledge of their bioavailability can be used to test whether "normal" concentrations could have been enriched by fertilizers to levels comparable with those measured in counterfeits. The approach used here is generalized as many factors influencing bioavailability and plant uptake are necessarily unknown, but the use of average values on a logarithmic plot (Figure 3) allows us to test whether the application of a particular fertilizer is likely to produce the

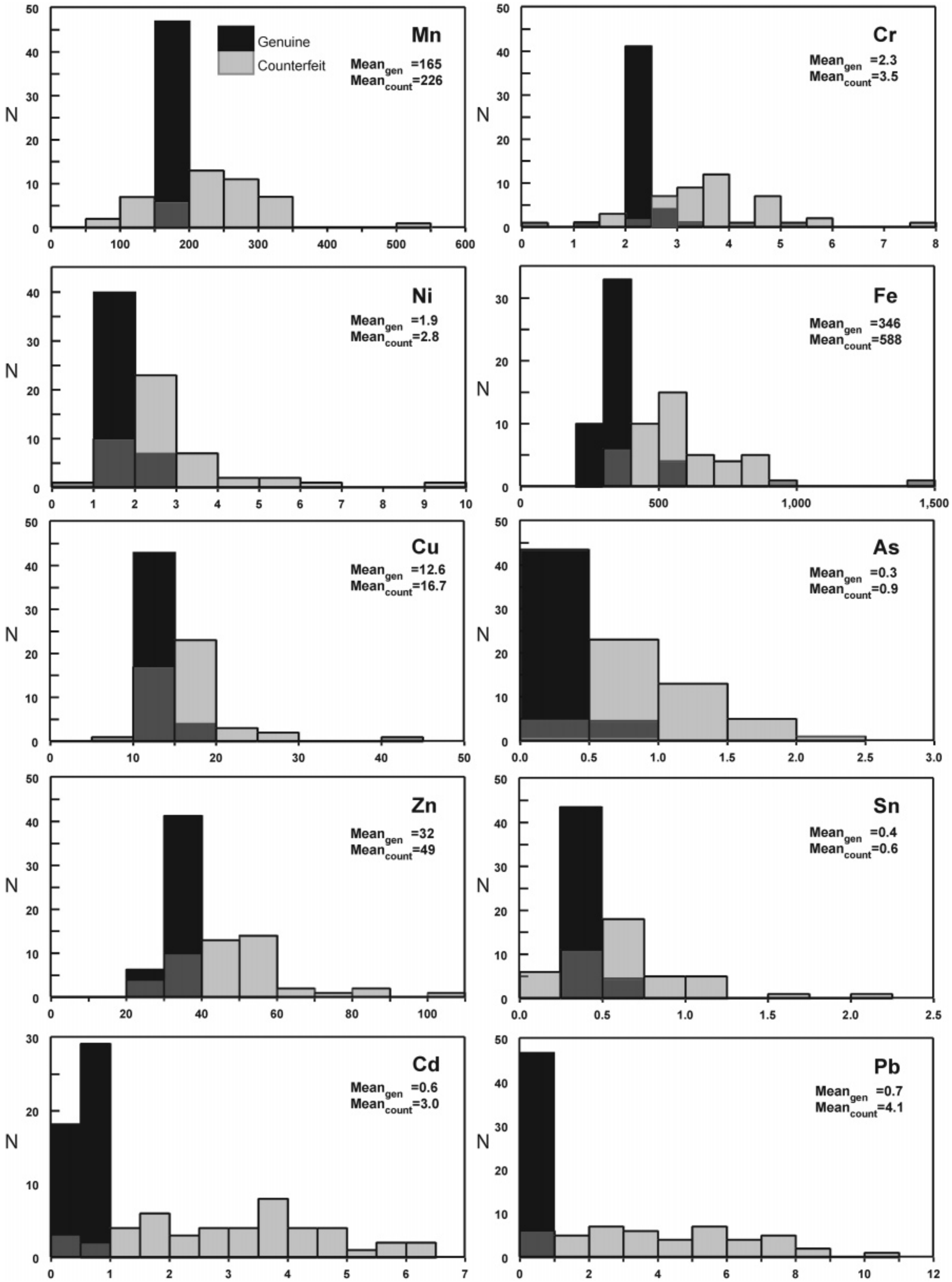

FIGURE 1. Histograms comparing the heavy metal contents of U.K. counterfeit cigarettes with their genuine equivalents for the elements Mn, Cr, Ni, Fe, Cu, As, Zn, Sn, Cd and Pb.

observed sense and magnitude of change. This is possible because the variance within genuine tobaccos is small as compared with the enrichment in counterfeits.

Heavy metal concentrations in fertilizers and tobaccos are compared using ratios to average continental crust (*35*) as this is the ultimate source of these metals (Figure 3). The plot also reveals changes in ratios of heavy metals as changes in slope between the two values. Thus two samples with parallel trends on such a plot have compositions in which the ratios (but not absolute values) of pairs of elements are similar. Average U.K. counterfeits show a trend parallel to that of their genuine equivalents but enriched in all analyzed heavy metals, by as much as half an order of magnitude in the cases of Cd and Pb.

Mean values for heavy metals in fertilizers applied in the U.K. (*36*) are used here as representative compositions.

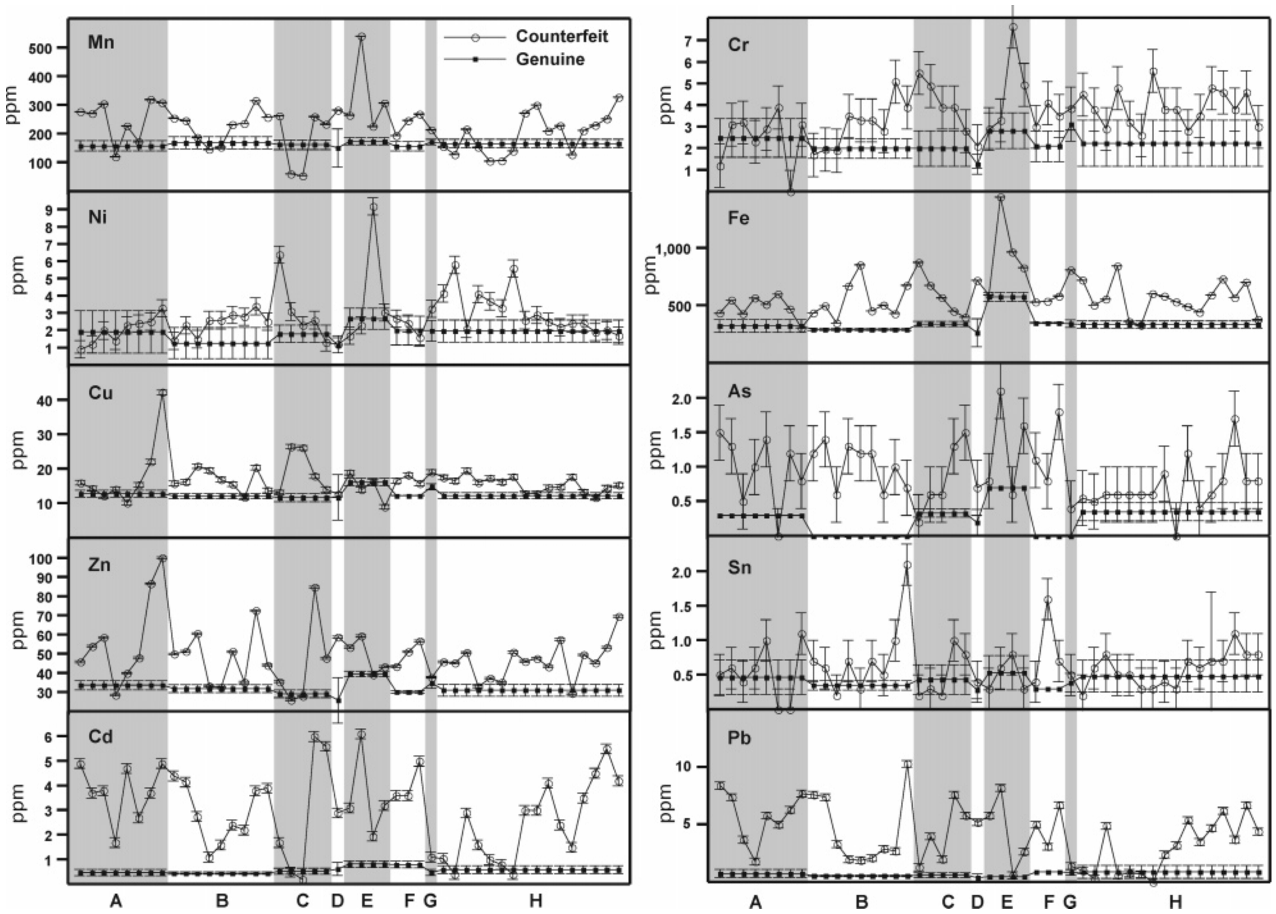

FIGURE 2. Like-for-like comparison of the mean concentrations of heavy metals in counterfeits with their genuine equivalents. Error bars are $\pm 2\sigma$ from the mean. A−H are codes for particular brands.

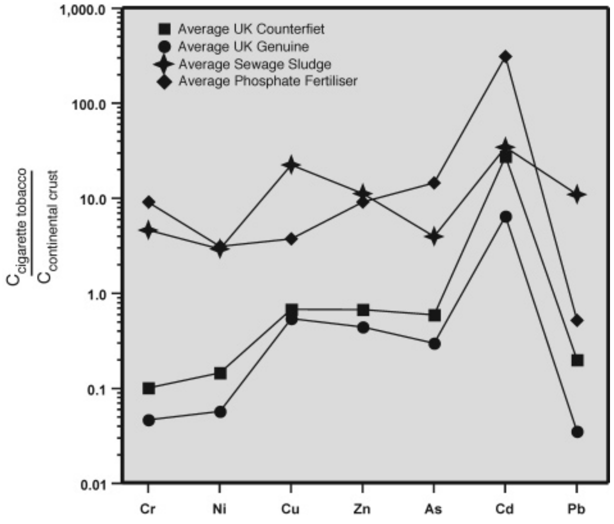

FIGURE 3. Mean values for selected heavy metals in average counterfeit cigarettes and average U.K. genuine equivalents compared with mean values for sewage sludge and phosphate fertilizers (*36*). Concentrations presented as ratios to average continental crust values (*35*).

Phosphatic fertilizers show a heavy metal trend remarkably parallel to that of U.K. average counterfeits (Figure 3). This trend is entirely consistent with a model in which tobacco used for U.K. counterfeit cigarettes is grown in soil heavily contaminated by average phosphate fertilizer. The enrichment in counterfeits is also consistent with a sewage sludge source of heavy metals, although the trend in Figure 3 is not as convincing as phosphate contamination. The similarity of Cd concentrations in the counterfeits and average sewage sludge can be explained by the very high phytoaccumulation rate of Cd in tobacco (i.e., the metal is being removed more efficiently from the fertilizer than the other metals). This graphical model suggests that application of either fertilizer (or both) could provide the additional heavy metal input seen consistently in U.K. counterfeit cigarettes. However these averages disguise wide ranges in the heavy metal concentrations of the sources. For instance, Kola (FSU) phosphate has <1 mg kg^{-1} Cd whereas that from the western United States may contain as much as 300 mg kg^{-1}. Such rocks are the basis of various high-P mineral fertilizers that acquire much of the heavy metals from the parental rock (*29*). Use of phosphatic sources rich in heavy metals is declining in the developed world due largely to regulation and application of best practice. This makes it more likely that these fertilizers find their way to less heavily regulated environments some of which are implicated in the cultivation of tobacco destined for the counterfeit industry. Similar arguments can be used in the application of sewage sludge, an excellent nitrate fertilizer, but which is now increasingly regulated because of its heavy metals, some of which are known to enter the food chain (*30*) and domestic and workplace environments (*34*).

The analysis of micronutrients and other trace elements cannot unambiguously distinguish between these likely anthropogenic inputs, nor can the concentrations of macronutrients such as Ca, K, and P. Phosphorus levels are not consistently higher or lower in counterfeits as compared with genuine equivalents, nor is there any strong correlation

TABLE 2. Nitrogen and Carbon Isotopes in a Subset of Counterfeit and Genuine Tobacco Samples

brand/label	type	δ^{15}N (‰)	δ^{13}C (‰)
OTL-1	tobacco leaf standard	+4.8	−23.0
VTL-1	tobacco leaf standard	+5.4	−24.4
KU-1R4F	cigarette standard	+3.9	−21.6
KU-1R5F	cigarette standard	+4.0	−24.4
B	genuine	+2.5	−24.2
D	genuine	+2.7	−22.8
E	genuine	+2.9	−25.7
F	genuine	+3.3	−24.0
H	genuine	+2.6	−25.5
A	counterfeit 2	+2.4	−25.4
A	counterfeit 3	+2.8	−25.1
B	counterfeit 9	+2.1	−18.3
B	counterfeit 10	+2.6	−22.5
B	counterfeit 11	+1.9	−21.4
B	counterfeit 12	+1.2	−22.5
B	counterfeit 13	+1.6	−23.0
B	counterfeit 15	+2.8	−22.0
C	counterfeit 18	+4.6	−24.5
D	counterfeit 23	+2.6	−20.4
E	counterfeit 25	+4.2	−26.4
E	counterfeit 24	+1.8	−24.4
G	counterfeit 31	+3.6	−21.4
H	counterfeit 32	+3.6	−24.9
H	counterfeit 33	+3.2	−26.0
H	counterfeit 34	+1.1	−26.4
H	counterfeit 35	+3.1	−21.6
H	counterfeit 36	+2.5	−26.1
H	counterfeit 37	+2.7	−26.0
H	counterfeit 39	+3.2	−25.8

between the heavy metals and P concentrations in the data set. Indeed such analysis is made difficult by other potential sources of contamination. Although elements such as Cr, Pb, As, and Cu are poorly accumulated from soils by crops such as tobacco, they are enriched in counterfeits. Thus we must also appeal to other sources of contamination, and soil and dust particles are well-known to stick to the rather adhesive tobacco leaf (*37*, *38*).

Stable Isotopes. Stable isotopes provide an independent means of testing between these inorganic and organic fertilizer inputs (*39*). Ratios of the various stable isotopes of carbon and nitrogen (expressed as δ^{13}C and δ^{15}N) are functions of both the plant species and their abiotic and biotic environments (*40*). Sewage- or manure-based nitrate fertilizers are significantly enriched in ^{15}N (δ^{15}N > +8‰) (*39*) as a consequence of removal of ^{14}N by digestive tracts. Further enrichment by the loss of ammonia can lead to values of δ^{15}N > 10‰ or even 20‰ (*41*). Heavy application of a fertilizer with this signature is likely to impart a detectably strong positive δ^{15}N signature to the soil, which in turn would strongly influence the plant δ^{15}N (*42*). This assumes that nitrogen in the organic fertilizer has not substantially reequilibrated with its environment. Indeed this is the basis of a successful approach to identifying the input of manure and/or sewage-based fertilizers in cannabis leaves, which has been suggested as a forensic tool for provenancing illegal crops (*43*). We adopted this approach for this study and also analyzed carbon isotopes as constraints on location and cultivation environment (*42*), given the unavailability of such information for the crops used to supply the counterfeiters.

Nitrogen and carbon isotopes were measured in tobacco removed from a large subset of counterfeit samples representing the full range of heavy metal concentrations, tobacco from their genuine equivalents, and a group of international reference standards. The results are presented in Table 2.

Caution must be exercised in the interpretation of nitrogen isotopes in plants. The combination of confounding factors (*44*), both gains and losses as well as within-plant fractionations, means that δ^{15}N in foliage and associated soils are only loosely correlated (*45*). Even the same soil source can show decadal variations in δ^{15}N of up to 2‰ (*45*, *46*).

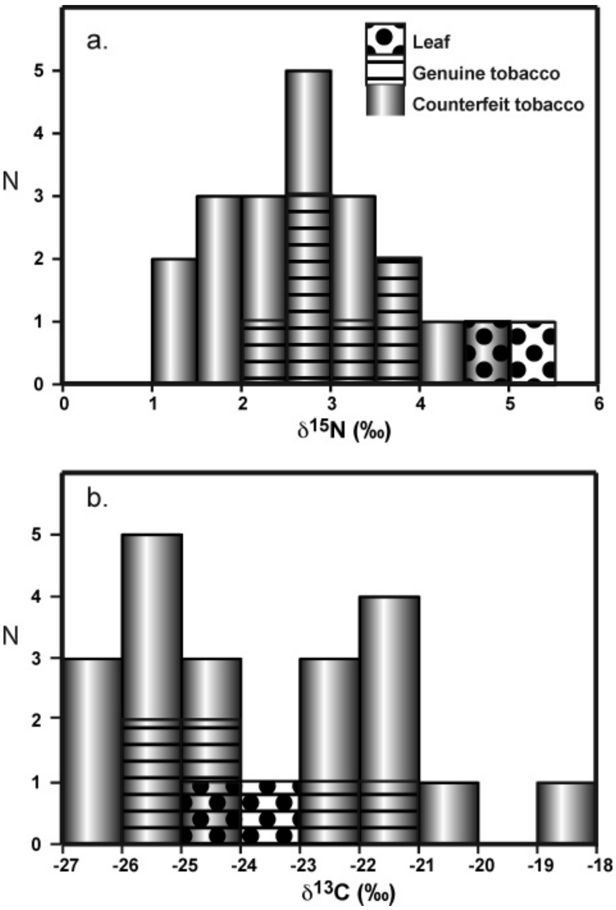

FIGURE 4. Histograms comparing tobacco leaf standards, genuine brands, and counterfeits for (a) δ^{15}N and (b) δ^{13}C.

Fractionation of nitrogen isotopes is influenced by various factors (*44*, *47*), but temperature and aridity (as mean annual temperature and mean annual precipitation) appear to be the main controls on both soil and plant δ^{15}N (*42*). The use of δ^{15}N for the purpose of identifying sewage sludge and manure inputs also assumes that their application to soils imparts an enrichment in ^{15}N that will persist for some time before equilibrating with the atmospheric and soil nitrogen reservoirs.

The range of foliar δ^{15}N in the natural environment is about −8 to +12‰ (*44*, *48*). Values for cigarettes tobaccos obtained in this study range from +1.1 to +5.4‰ (Table 2 and Figure 4a). It is significant that the highest values are found in the CTL tobacco leaf standards; all other samples are cigarettes, which contain stalk material as well as leaf. Loss of volatiles from the leaves may explain their heavier isotopic ratios. Genuine cigarette tobacco has a fairly narrow range between +2 and +4‰ whereas counterfeits are more widely dispersed but with the same mode (Figure 4a).

These unexceptional values do not suggest substantial amendment of soil by manure (δ^{15}N range of +10 to +22‰) or sewage sludge, at least not on the scale detected for cannabis (*43*). It is possible that the values have been changed post-harvesting, for instance during the aging and fermentation of tobacco for several months prior to production. This normally results in some decrease in total nitrogen (*49*) through loss of ammonia and other nitrogenous compounds (*50*), implying that the *harvested* product would be relatively depleted in ^{15}N.

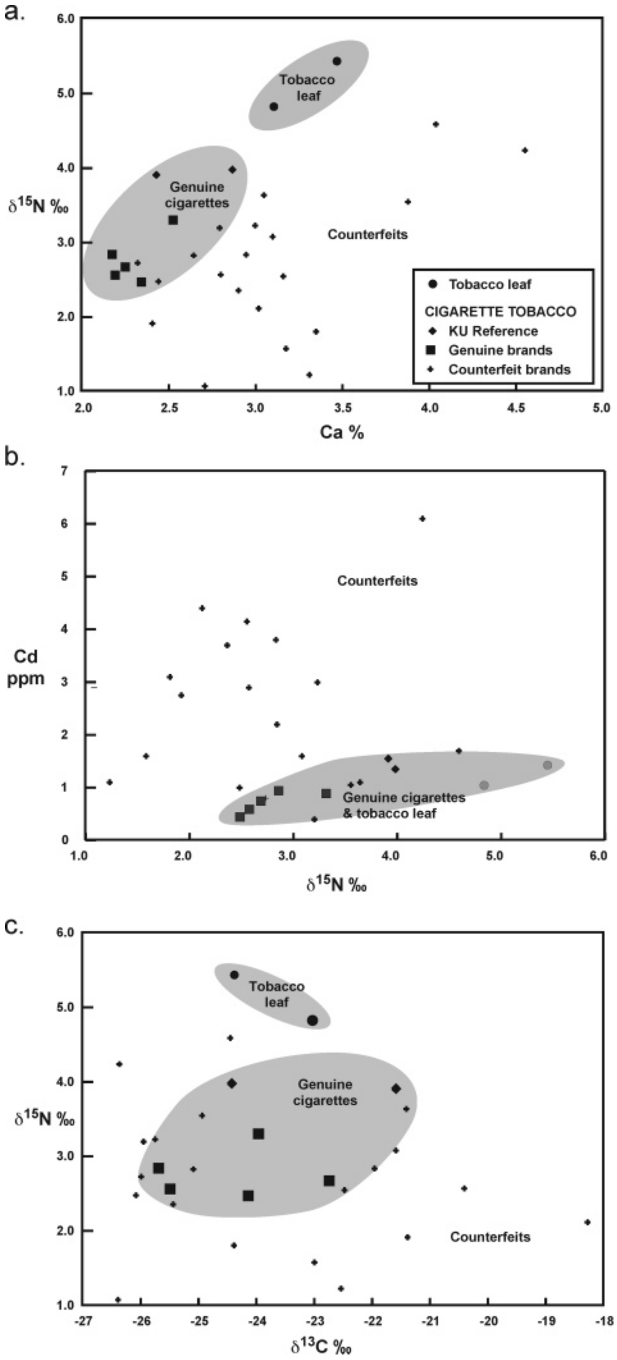

FIGURE 5. Scatterplots comparing counterfeit compositions with genuine brands and reference standards for (a) δ^{15}N and Ca, (b) δ^{15}N and Cd, and (c) δ^{15}N and δ^{13}C.

The concentration of inorganic nutrients in tobacco is largely attributable to soil and fertilizer practices (*49*). Figure 5a shows that tobacco leaf is significantly richer in both Ca, one of the most important macronutrients and ^{15}N. The pattern for counterfeits is similar but more dispersed and displaced to higher calcium and/or lower δ^{15}N. Both controlled environment studies and global reviews of variations in δ^{15}N indicate that drier conditions favor ^{15}N (*42*−*44*), suggesting that the crops supplied to the genuine manufacturers were grown in drier climates or were not as extensively irrigated. Alternatively (or additionally) the illicit crop may have been subjected to greater lime input, either naturally through location in limestone terrain or by application of lime fertilizer. Lime and limestone will increase soil pH and consequently reduce the bioavailability of some heavy metals including Cd, whereas the plants used in these counterfeits are generally enriched in these metals, so this is an unlikely explanation.

Nitrogen isotopes have not provided strong evidence for sewage sludge as the primary source of the heavy metal enrichments in counterfeit cigarettes, although they do not negate this possibility as data for well-constrained cultivation settings are not yet available. A significant positive correlation between Cd and δ^{15}N is present in genuine samples ($r = +0.74$, Figure 5b) perhaps suggesting a causal relationship, but no such association can be discerned among the counterfeits. A similar effect is seen for other heavy metals analyzed, although not for Cu. The stable isotopes of carbon are less useful as source indicators than nitrogen because plants gain the bulk of their carbon from the well-mixed atmospheric reservoir and the main fractionation is associated with photosynthetic fixation which discriminates against the heavier isotope (*40*, *51*). C3 plants, which include *N. tabacum*, range in δ^{13}C from −22 to −34‰ (*52*). Environmental factors influence plant δ^{13}C by affecting the ratio between intercellular and atmospheric CO_2. High soil moisture and air humidity tend to be associated with plants with lighter isotopes (*43*) (i.e., more negative values of δ^{13}C), and as such the isotope ratio within a given species gives some indication of prevailing climatic conditions.

The observed range of δ^{13}C is −18.3 to −26.4‰ (Table 3 and Figure 4b) although the heavy value seems aberrant and most samples lie in the range −21.4 to −26.4, at the heavy end of the natural range for C3 plants. Tobacco can tolerate a wide range of climates, including wide variations in humidity (*53*), and this may in part explain the range in δ^{13}C.

Only a few reported measurements of δ^{13}C in bulk tobacco or tobacco leaf were found in the literature, quoting values of ~30‰ (*54*, *55*). Significant fractionation occurs between upper, middle, and lower leaves of NH_4^+-fed plants from about −27 to −30‰ (*56*), and a very similar pattern has been shown in cannabis (*43*). These ratios are lighter than any measured in this study and may reflect the artificial cultivation environment of these tobacco plants with regular watering and nutrient treatments. Perhaps more likely, the process of aging and fermentation generates carbon dioxide (*50*), which (in an analogous way to ammonia and nitrogen isotopes described above) will tend to remove the lighter isotope in the volatile phase. The consequence is probably that commercial cigarette tobaccos acquire less negative δ^{13}C values through this process), although the extent is not known.

Other than the studies quoted above, there is a dearth of information on the ratios of these stable isotopes in the tobacco plant, and there are few constraints on the results obtained in this study. As discussed above, water availability influences both δ^{13}C and δ^{15}N relationships. Wet climates and/or well-irrigated crops tend to produce lower (lighter) values of both carbon (*51*) and nitrogen (*44*) isotopes. If water availability was the primary control on these isotopic ratios, then a positive correlation would be predicted, but inspection of Figure 5c indicates that is no such correlation exists.

Sources of Enriched Heavy Metals in Illicit Tobacco Production. Various factors influence metal enrichments in tobacco, including soil type, pH, genotype, stalk position, and pesticides (*3*), and fertilizers have also been implicated in such heavy metal enrichment (*57*). Both sewage-based nitrate and phosphate fertilizers can provide the necessary heavy metal enrichments. Nitrate fertilizer has to be applied carefully in the growing of tobacco, and for Oriental tobacco cultivation it is not normally applied at all (*58*). Unlike the clear outcome of a study on illicit cannabis crops (*43*), no clear signature of sewage sludge or animal manure sources of nitrate was found in the stable isotopes of counterfeit tobaccos. On balance, phosphate fertilizer would seem to be the more likely source, and heavy metal enrichment in tobacco leaves in Tanzania has been attributed to this source (*59*). The difference between genuine and counterfeit tobacco may thus reflect differences in regulatory controls on heavy metal levels and limitations on fertilizer application between the developed and the developing worlds.

Health Implications for Smokers of Heavy Metal-Enriched Counterfeit Cigarettes. Such heavy metal enrichment would be of little concern if there was no transfer to the lungs and to other organs via the bloodstream. However, experiments on the partitioning of heavy metals between smoke (both mainstream and sidestream), ash, and filters indicate that a substantial proportion of some metals in tobacco reaches the lungs of smokers during the smoking process. Cadmium has been found in several studies consistently to transfer into the smoke phase (*6*, *18*, *25*, *26*, *28*, *60*−*67*), which coupled with the fact that the tobacco plant is particularly efficient in accumulating Cd from the soil and translocating most of the metal to the leaves (*68*) makes this element the prime focus for particular investigation for any potential toxic effects. This is not intended to diminish the importance of other elements; lead and others merit further assessment.

The toxicity of cadmium and other heavy metals to humans is well-documented, and smoking is a significant contributor (*8*). Various estimates of the proportion of cadmium in tobacco that is liberated into the smoke phase have been obtained by analyzing tobacco, paper, and filter prior to smoking, and ash, cigarette butt, mainstream smoke (MS), and sidestream smoke (SS) during and after smoking. These generally show that both MS and SS carry significant cadmium (*3*, *6*, *25*, *28*, *60*, *62*, *66*) typically as particulates (*62*) and filters appear to be inefficient at reducing cadmium levels in MS (*60*). It has been suggested that smoking 40 cigarettes per day provides about twice as much cadmium as that present in food (*2*). Habitually smoking counterfeit cigarettes will significantly increase that dose and add to the hazard from toxic heavy metals faced by the smoker.

The principal factors influencing the effect of cadmium on human organs are intake (dietary and smoking) and body mass, which are reflected in the current World Health Organization (WHO) weekly tolerable intake of 7 μg per week per kilogram of body weight. On this basis the effect is likely to be most pronounced in low body mass individuals such as children and the under-weight adults whose smoking and dietary habits maximize their cadmium intake. Foods such as potatoes, grain, and cereal (especially rice) deliver relatively high quantities of Cd, whereas meat delivers less and legumes and fruits deliver very much less (*19*). Detailed evaluation of factors influencing human exposure and toxicology of cadmium and other metals is available in major monographs (*69*, *70*). Two groups, namely, children and undernourished adults, are well represented among the lower income groups who habitually purchase counterfeit cigarettes and whose diet can be rich in the very foods known to deliver more Cd to the body. Considering these lifestyle features as a whole suggests that more research should be undertaken into assessing whether these groups face any risks from the effects of high cadmium intake (and other heavy metals) over the longer term.

Cadmium, not being an essential nutrient, is harmful to the body in several different ways, most notably by renal tubular dysfunction (*19*). Recent studies indicate that a threshold of Cd in urine of 10 μg/g creatinine must be exceeded before this disorder becomes evident in the population (*71*). It is unlikely that smoking high Cd tobacco will cause many to exceed this threshold from this source alone. However low-level exposure such as that from combined dietary and smoking sources can result in unrecognized but potentially significant burdens on health (*72*, *73*). Some recent studies have raised the possibility that levels of cadmium intake very much lower than that recommended by the WHO may have important toxic and genetic effects (*74*−*76*) and that levels of Cd and Pb well below recommended exposure limits can still increase the risk of peripheral arterial disease (*77*). Another concern is that other heavy metals may also be entering the lungs and bloodstream, and the effects may be additional or even synergistic with cadmium (*78*), but experimental transfer coefficients between tobacco and smoke are not presently well-characterized for many heavy metals. If any of these low-dose effects proves to cause significant harm, then the role of enhanced levels of Cd and other heavy metals in cigarette smoke becomes rather more significant than currently appreciated.

Further Implications. The purchase of counterfeit cigarettes is often regarded as a victimless crime, with evasion of tax being the only misdemeanor. As well as loss of considerable government revenue, there are important social implications. Organized criminal gangs manage the manufacture and distribution of counterfeits, and increasing resources are having to be diverted to combating this activity. The main purchasers of counterfeit cigarettes are dominantly those on low incomes, either young people who then become addicted to smoking or the socially disadvantaged for whom so many other factors impact negatively on their state of health that the addition of another factor is potentially very serious. The extent of the U.K. market share now claimed by counterfeits means that an issue once considered marginal is rapidly becoming a major problem. The health risks described above as well as social implications means that early awareness of these issues is important if remedial action is to have significant impact.

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