1 **Adsorption Capacity of Multiple DNA Sources to Clay Minerals and Environmental**
2 **Soil Matrices Less Than Previously Estimated**

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16 **Abstract**

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18 The cultivation and consumption of transgenic crops continues to be a widely debated
19 topic, as the potential ecological impacts are not fully understood. In particular, because
20 antibiotic resistance genes (ARGs) have historically been used as selectable markers in
21 the genetic engineering of transgenic crops, it is important to determine if the genetic
22 constructs found in decomposing transgenic crops persist long enough in the environment
23 and if they can be transferred horizontally to indigenous microorganisms. In the present

study, we address the question of persistence. Others have also estimated the DNA adsorption capacity of various clays, but have done so by manipulating the surface charge and size of particles tested which may overestimate sorption and underestimate the DNA available for horizontal transfer. In the present study, isotherms were generated using model Calf Thymus DNA and transgenic maize DNA without surface modification. Montmorillonite, kaolinite, and 3 soil mixtures with varying clay content were used in this study. The adsorption capacity of pure montmorillonite and kaolinite minerals was found to be one to two orders of magnitude less than previously estimated likely due to the distribution of clay particle sizes and heteroionic particle surface charge. However, it appears that a substantial amount of DNA is still able to adsorb onto these matrices (up to 200 mg DNA per gram of clay) suggesting the potential availability of free transgenic DNA in the environment may still be significant. Future studies should be conducted to determine the fate of these genes in agricultural soils.

Keywords: genetically modified crops, DNA adsorption, Langmuir isotherm

Introduction

Acquisition of antibiotic resistance has increased dramatically in both clinical and environmental bacterial populations over the past several decades and has largely been attributed to the widespread use of antibiotics for medical and farming applications¹⁻⁴. An upsurge in the number of bacteria that have acquired immunity to a single antibiotic as well as multi drug resistant (MDR) bacteria has been noted in a wide array of environments ranging from hospital settings to wastewater treatment plants (WWTPs) and groundwater⁵⁻⁶. In particular, antibiotic resistance genes (ARGs) for tetracyclines and β -lactamases have been shown to have some of the highest rates of increase among soil bacteria⁷⁻⁹. Some forms of β -lactam resistance from the CTX-M family appear to have arisen before the current medical crisis, suggesting that some bacteria may have acquired resistance from sources other than exposure to antibiotics⁹. While the causes underlying these trends have been investigated thoroughly in a clinical setting, little work has been done to estimate the contribution of ARGs derived from non-traditional sources such as those from transgenic crops.

Several lines of genetically modified (GM) crops are known to contain one or more ARGs including β -lactamase and neomycin resistance genes. These genes have been traditionally used as selection markers during the plant cell transformation process, and are most commonly found in first and second generation GM crops¹⁰⁻¹³. With more than 159.9 million hectares of GM canola, maize, soy, and cotton planted globally in 2012¹⁴, the risk of these genes entering into the environment is noteworthy. Several lines of

transgenic maize, including Bt176, contain the *Bla* gene, encoding resistance to penicillins, cephamycins, and carbapenems¹⁵. However, it is worth noting that these genes are only contained in a fraction of GM crop lines and ARGs are being used as selection markers in GM crops less and less frequently. However, as these same β -lactam antibiotics are still used by the medical community to treat a variety of Gram-positive and Gram-negative bacterial infections, it is important to understand how the current and historical use of transgenic crops containing ARGs may have contributed to the increasing rates of antibiotic resistance among soil bacteria. To this end, an accurate assessment of how much transgenic DNA is entering into the environment and persisting must be obtained.

It has been well established that nucleic acids can persist in soils for a significant amount of time, especially in soils with elevated clay content¹⁶⁻¹⁸. Previous studies have estimated the capacity of several pure clay materials¹⁹⁻²¹ as well as environmental soils and sediments^{17,22} to adsorb model DNA such as calf thymus or herring sperm DNA. Some of these experiments were unable to find a maximum adsorption capacity, citing values in excess of 9,000 μg calf thymus DNA/g dry clay or 30,000 μg herring sperm DNA/g clay¹⁸. They have also demonstrated that the resulting DNA-clay complexes have the ability to shield bound DNA from significant DNase activity^{21,23}, likely increasing DNA persistence in environmental soil. However, many of the studies that examined the DNA adsorption capacity of pure clay minerals calculated the adsorption capacities of specific clays and soils after separating particles based on size, often only using particles $< 2 \mu\text{m}$, and making clays homoionic to Ca^{2+} , Na^{+} , or Mg^{2+} . This pretreatment is not relevant to

any known environmental condition and has likely resulted in an overestimation of the DNA adsorption capacity of tested soils and clays. These studies have also only measured total DNA adsorption to clay surfaces making no distinction between ssDNA and dsDNA, which may have varying microbial transformation potential. Thus, in the present study, several adsorption isotherms were constructed to evaluate the natural ability for pure kaolinite, a 1:1 clay mineral, and Montmorillonite, a 2:1 clay mineral, as well as three environmental soil samples with varying clay content to adsorb model calf thymus DNA and transgenic maize DNA. The resulting isotherms were compared to past literature in order to determine each mineral's natural ability to adsorb DNA, without the introduction of particle charge or size biases. The adsorption capacity of calf thymus DNA was compared to transgenic maize DNA to test the effect of source DNA GC content on the formation of DNA-clay complexes. Finally, the adsorption potential of transgenic maize DNA was further tested on three environmental soil matrices.

Materials & Methods

Clay Minerals and Soils. Montmorillonite, kaolinite, and acid washed sand were obtained from Sigma (St. Louis, MO). Sandy loam, clay loam, and silt loam soil matrices (Table 1) were obtained through Sands and Soils (Durham, NC). Soil mixtures were homogenized and ground to pass through an 80 mesh sieve²². In addition, the pH of kaolinite and montmorillonite was found to be 6.82 and 4.01, respectively. All characterization of soil physicochemical properties was performed by the North Carolina Department of Agriculture and Consumer Sciences Division in accordance with standard protocols.

Table 1. Physico-Chemical Properties of Soils Used for Adsorption Isotherms

Soil Type	% Clay	% Humic Matter	CEC (mequiv/100g)	pH
Clay Loam	39.0	0.09	6.7	5.3
Sandy Loam	12.7	0.04	2.0	5.5
Low Clay Soil	3.0	0.22	5.8	5.2

DNA. Calf thymus DNA (Sigma-Aldrich, St. Louis, MO) with an approximate GC content of 41.9% was obtained and resuspended in molecular grade nuclease-free water to a stock concentration of 500 µg/mL. High quality genomic DNA from transgenic maize with an average GC content of 42.3-55.4%²⁴ was isolated using the following optimized CTAB isolation protocol. First, 500 µL CTAB Buffer (100mM Tris-HCl (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 1% polyvinylpyrrolidone, 2% hexadecyl trimethyl-amonium bromide (CTAB) (w/v), and 0.5% β-Mercaptoethanol (v/v)) was added to 100 mg maize biomass and incubated at 60°C for two hours. Samples were removed from heat and 1.0 µL RNase A (10 mg/mL) was added to the mixture and incubated at 37°C for 30 minutes to remove RNA and increase the accuracy of spectrophotometric measurements. Seven hundred µL phenol:chloroform:IAA was added, tubes were vortexed briefly and centrifuged at 13,000x g for 60 seconds. This step was repeated until no debris could be seen at the interface. Then, 700 µL chloroform was added to the aqueous phase and tubes were centrifuged at 13,000x g for 2 minutes. The aqueous phase was transferred to a new tube and 0.08 volumes of cold 5 M ammonium

acetate and 0.54 volumes of isopropanol were added, mixed by inversion, and incubated on ice for 45 minutes. DNA was then purified by ethanol precipitation and the resulting pellet was resuspended in 50 μ L TE Buffer. DNA concentration and quality were determined using a Qubit fluorometer 2.0 for dsDNA only and NanoDrop spectrophotometry for total DNA. As NanoDrop instruments are known for significantly overestimating DNA concentrations, the degree of DNA overestimation was calculated using a series of DNA solutions with known DNA concentrations. The authors found the NanoDrop to overestimate total DNA by an average factor of 2.957. Thus, the raw NanoDrop readings obtained by the authors were divided by a factor of 2.957 to calculate the true concentration of total DNA (μ g/mL)²⁵. The concentration of ssDNA was estimated by subtracting dsDNA Qubit concentrations from total DNA NanoDrop measurements. Only extracted DNA with a 260/280 of 1.8-2.0 and a 260/230 of 2.0-2.2 were used for adsorption isotherms²¹. RNA contamination in both DNA sources was determined to be negligible. This was assessed using the Qubit RNA HS kit (Thermo Fisher Scientific).

Adsorption Isotherms. Adsorption isotherms were constructed using a batch slurry method similar to that developed by Bainin *et al.*²⁶ using siliconized 15 mL glass centrifuge tubes. A total weight of 0.5 g clay or soil was added to 1.0 mL nuclease free dH₂O containing a DNA concentration ranging from 0 to 150 μ g/mL. Suspended DNA was not found to be significantly denatured by the lack of ions over the course of the isotherm experiments. Calf Thymus DNA was used as a source of DNA material for kaolinite and montmorillonite adsorption isotherms only. Because Calf Thymus DNA has

151 been used historically as a DNA source in adsorption experiments it will allow direct
152 comparisons between the clay adsorption findings in this study and those of older studies.
153 Transgenic maize DNA was used as a DNA source for both pure clay isotherms and all
154 three environmental soil isotherms because of its increased environmental relevance and
155 potential to harbor antibiotic resistance genes. All isotherms were performed in
156 quadruplicate. Tubes were mixed at 80 rpm for two hours and then centrifuged at 6,000x
157 g at 4°C for 30 minutes. Supernatants were collected and centrifuged again at 11,000x g
158 4°C for 20 minutes. The DNA concentration in the resulting final supernatant was
159 analyzed using a NanoDrop and Qubit 2.0 according to manufacturer instructions for
160 total DNA and dsDNA concentrations, respectively.

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162 *DNase Detection.* Soil substrates and pure clay minerals were tested for the presence of
163 DNase proteins using DNase Alert kits (IDT, Coralville, IA). As no standard method for
164 quantifying DNase proteins in soils matrices were known to the research team samples
165 were prepared in multiple ways to optimize the kit-based protocol and determine the best
166 method for detecting DNases. Soils were pretreated using the following protocols: (1)
167 0.025g of soil was added to 200 μ L dH₂O and was shaken at 120 rpm at room
168 temperature for 30 minutes. Samples were collected every 5 minutes and centrifuged at
169 13,000x g for 10 minutes and supernatants were collected and measured for the presence
170 of DNases according to kit manufacturer instructions. (2) 0.025 g of soil was added to
171 200 μ L dH₂O and was shaken at 120 rpm at room temperature for 30 minutes. This slurry
172 was measured for DNases every 5 minutes without centrifugation. Positive and negative
173 controls were used for each sample set. All tests for the presence of DNases in tested

clays and soils using the DNase Alert kit were performed in triplicate and found to be negative. Therefore, we assumed that the relative contribution of DNases to the sorption values obtained in this study were minimal and no adjustments were made.

Statistical Analysis. The resulting concentrations were evaluated using the Langmuir equation (Equation 1)

$$Q=Q_{MAX}K_L C_e/(1+ K_L C_e) \quad (\text{Equation 1})$$

where Q_{MAX} is the maximum adsorption capacity ($\mu\text{g/g}$), Q is the adsorbed DNA concentration ($\mu\text{g/g}$), C_e is the DNA concentration in solution at equilibrium ($\mu\text{g/mL}$), and K_L is the Langmuir constant ($\text{mL}/\mu\text{g}$). Each isotherm was linearized as C_e/q_e vs. C_e to determine the Langmuir constants and coefficient of determination (R^2) values. Statistically significant differences among isotherms were assessed using one-way analysis of variance (ANOVA) and paired t-tests.

Results

Clay Adsorption Capacity. The adsorption trends of calf thymus DNA was tested using untreated kaolinite and montmorillonite clays (Figure 1). For all tested materials, the adsorption capacities were reached when the adsorbed DNA concentrations (Q_e) failed to increase while the DNA concentration remaining in the supernatant (C_e) continued to increase suggesting that all available adsorption sites on the clays had been saturated. In general, calf thymus DNA adsorption occurred rapidly onto both clays and continued to

increase. Adsorption rates started to decrease at approximately 200 μg total DNA/g dry kaolinite and 40 μg total DNA/g dry montmorillonite. The adsorption of dsDNA was favored over ssDNA for both clays, with a higher observed fraction of ssDNA in the supernatants remaining after equilibrium had been reached. A statistically significant difference also was detected between the adsorption capacities of untreated montmorillonite and kaolinite clays for calf thymus DNA ($p=0.047$)

Figure 1: Adsorption of model Calf Thymus DNA to (A) pure Kaolinite clay and (B) pure Montmorillonite clay. Q_e : amount of DNA adsorbed (μg DNA per g dry clay). C_e : Concentration of DNA remaining in solution after equilibrium had been reached (μg DNA per mL dH_2O). Square data points correspond to total DNA (dsDNA + ssDNA) measurements while circle data points correspond to dsDNA only.

The adsorption of transgenic maize DNA was also tested (Figure 2). As with the calf thymus DNA isotherms, the adsorption of transgenic maize DNA onto pure kaolinite and montmorillonite started rapidly. Kaolinite adsorption trends continued to increase up to

40 μg total DNA/g kaolinite, where the adsorption rate began to lessen. Montmorillonite adsorption of DNA saturated at approximately 30 μg DNA/g montmorillonite.

Figure 2: Adsorption of transgenic maize BT176 DNA (A) pure Kaolinite clay and (B) pure Montmorillonite clay. Q_e : amount of DNA adsorbed (μg DNA per g dry clay). C_e : Concentration of DNA remaining in solution after equilibrium had been reached (μg DNA per mL dH_2O). Square data points correspond to total DNA (dsDNA + ssDNA) measurements while circle data points correspond to dsDNA only.

The adsorption trends of single and double stranded transgenic maize DNA were similar to those observed when testing the adsorption of calf thymus DNA, as there was a significant difference in the adsorption capacities of each mineral ($p=0.043$). Though it was only marginally statistically significant, there was also a slight difference in the adsorption of calf thymus DNA and transgenic maize DNA onto kaolinite particles ($p=0.062$), with more Calf Thymus DNA able to adsorb. However, no significant difference was measured between the adsorption of the two DNA sources on montmorillonite ($p=0.34$)

Soil Adsorption Capacity. Transgenic maize DNA had limited ability to adsorb to the low clay soil, reaching a saturation plateau by the addition of an initial concentration of 5 $\mu\text{g/mL}$ (Figure 3). Adsorption trends for ssDNA were similar to those of dsDNA for this soil matrix. Sandy loam soil adsorption increased in a nearly linear fashion and leveled off slowly. Clay loam soil had the most rapid adsorption of all tested environmental soil matrices, though it still was considerably slower than the rates associated with kaolinite and montmorillonite. Adsorption of transgenic maize DNA rapidly increased until it reached a plateau at more than 60 $\mu\text{g/g}$. For both sandy loam and clay loam soil matrices, adsorption of dsDNA was again favored over the adsorption of ssDNA.

Figure 3: Adsorption of transgenic maize Bt176 DNA (A) minimal clay, (B) sandy loam, and (C) clay loam soils. Q_e : amount of DNA adsorbed (μg DNA per g dry clay). C_e : Concentration of DNA remaining in solution after equilibrium had been reached (μg

DNA per mL dH₂O). Square data points correspond to total DNA (dsDNA + ssDNA) measurements while circle data points correspond to dsDNA only.

The three tested environmental soils offer significantly different adsorption potentials for transgenic maize DNA, as determined through a one-way ANOVA. When analyzed individually using a paired t-test, there was a statistically significant difference between transgenic maize DNA adsorption to low clay soil and sandy loam soil ($p=0.033$), low clay and clay loam soils ($p=0.032$), and sandy loam and clay loam soils ($p=0.021$). The Langmuir adsorption constant (K_L) and maximum adsorption capacity (Q_{MAX}) values for all isotherms can be found in Table 2.

Table 2: Isotherm properties for all tested soil and DNA sources. The Langmuir adsorption constant K_L was determined experimentally and calculated using the Langmuir isotherm equation. The R^2 value quantifies the correlation strength between the linearized C_e/Q_e vs. C_e values and the overall suitability of the Langmuir Isotherm model for each isotherm.

Soil Matrix	DNA Source	K_L (mL/ μ g)	Q_{MAX} (μ g/g)	R^2
Kaolinite	Calf Thymus	.0058	243.9	0.91995
Montmorillonite	Calf Thymus	0.258	48.78	0.99981
Kaolinite	Transgenic Maize	.0058	55.56	0.99995
Montmorillonite	Transgenic Maize	.0229	28.74	0.99801
Minimal Clay	Transgenic Maize	8.560	0.805	0.04821

Sandy Loam	Transgenic Maize	10.83	6.064	0.93823
Clay Loam	Transgenic Maize	0.9705	73.52	0.97056

A small amount of DNA, approximately 0.8-3.1 $\mu\text{g/mL}$, was found to be present in control supernatants for both the low clay and sandy loam soils, and appears to be highly correlated to the amount of organic matter present in the soils (Figure 4).

Figure 4: DNA found in the supernatants of control adsorption isotherms as a function of the percent humic matter (HM) in each soil type. Hollow circle data points correspond to sandy loam soils (0.04% HM), Black squares correspond to clay loam soils (0.09% HM), and dashed data points correspond to low clay soils (0.22% HM). DNA found in control supernatants appeared to be highly correlated to levels of humic matter ($R^2=0.9938$).

For these samples, the amount of DNA present in the control supernatants after equilibrium was subtracted from the amount of DNA present in experimental

supernatants to get a final clay-adsorbed DNA for each treatment. The amount of DNA present in control supernatants was highly correlated to the amount of organic matter present in each soil ($R^2=0.9938$), with sandy loam having the lowest concentration of DNA and sandy soil having the highest concentration of DNA.

Discussion

While the correlation between high adsorptive capacity and higher soil clay content was expected, the difference in adsorption between kaolinite and montmorillonite was not. This disparity is particularly noticeable when comparing the adsorption of calf thymus DNA to the two clay minerals. One explanation for this disparity is the difference in mineral structure between these two clays. Kaolinite ($\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$) is a 1:1 clay mineral and is composed of one tetrahedral sheet bound to one octahedral sheet. The outer surface of the tetrahedral sheet is bound by O^{2-} atoms, while the outer surface of the octahedral sheet is bound by OH^- groups. The OH^- groups likely provide ample adsorption sites for the phosphate-based DNA backbone to become bound, either through O^{2-} substitution or the formation of an additional hydrogen bond between the kaolinite OH^- group and the PO_4^{2-} of the DNA²⁷. Montmorillonite $((\text{Na},\text{Ca})_{0.33}(\text{Al},\text{Mg})_2(\text{Si}_4\text{O}_{10})(\text{OH})_2 \cdot n\text{H}_2\text{O})$ on the other hand is a 2:1 clay mineral consisting of two tetrahedral sheets sandwiching one octahedral sheet. Unlike kaolinite, the top and bottom layers of this mineral consist entirely of O^{2-} atoms²⁸. The mechanisms available for free DNA to adsorb onto montmorillonite is likely limited to O^{2-} substitution between the O^{2-} of the tetrahedral sheets and the PO_4^{2-} groups of the DNA. This process

may be less energetically favorable, making adsorption more difficult. Similarly, transgenic maize DNA displayed a higher affinity for adsorbing to the surface of kaolinite over montmorillonite minerals. The relatively high percentage of GC content in maize genomic DNA may account for this discrepancy in adsorption capacity. The different C-G nucleotide structures may have a different binding affinity for kaolinite than A-T structures. Thus, this may have contributed to the discrepancies in adsorption capacities for these different DNA sources, though further studies are necessary to determine the exact mechanisms responsible.

The systems tested appeared to have a slight preference for the adsorption of dsDNA over ssDNA, as ssDNA was much more common in the final isotherm supernatants containing unbound DNA. The relative proportion of ssDNA and dsDNA used in the isotherm experiments may be dependent upon the DNA extraction method used and, thus, may vary from method to method. However, because only one method was used to extract transgenic maize DNA, this ratio is expected to be consistent in our experiments. The structural nature of the bound DNA (i.e, ssDNA vs. dsDNA) may be important as it may affect the ability of bound DNA to be taken up by soil bacteria and functionally integrated into bacterial genomes. For example, dsDNA may be easier for bacteria to integrate into their genomic DNA through homologous recombination or a similar mechanism as ssDNA would have the added difficulty of finding a complementary region to adhere to. Others have shown that the uptake efficiency of ssDNA is 200 fold lower than that of dsDNA of a comparable length and sequence homology²⁹.

This may have downstream effects on transformation rates in natural systems. Transformation is conventionally defined as the uptake of foreign DNA in the environment by a bacterium and the integration of that DNA into the genome of the host bacterium the process of homologous recombination. More than 60 bacterial species distributed among multiple taxonomic groups are known to display natural competence³⁰, and most of these species contain homologous recombination genes and pathways that are highly similar to one another³¹. One of the most well studied pathways is the SOS pathway and the RecA protein involved in this system. As dsDNA is taken up by competent bacteria and transported across the cell membrane through type IV pili and type II secretion systems³¹⁻³³, nucleases degrade one strand thereby converting it into ssDNA. Once ssDNA reaches the cytoplasm, the DprA protein transports the ssDNA to RecA proteins within the cell³⁴⁻³⁵. RecA binds to ssDNA, forming a RecA-DNA complex, and searches for an area of high sequence similarity on the dsDNA of the bacterial genome. RecA then catalyzes several reactions to complete the process of recombination, integrating the foreign ssDNA into the bacterial genome³⁴. This process is slightly altered between Gram positive and Gram negative bacteria, as their cell membrane structures vary significantly. However, most competent bacteria share the same general RecA-dependent transformation process as well as highly similar proteins³⁶.

Because dsDNA is required to be converted into ssDNA within the cell before homologous recombination can occur, it would be logical to assume that ssDNA would be preferred for transformation events. However, Miao *et al.*²⁹ found that dsDNA is preferentially taken up over ssDNA; this may have evolved as a defense mechanism

against single-stranded viral DNA. In addition, it is likely that most ssDNA in the environment is less stable and would be degraded by DNases and unable to be taken up by competent bacteria. GC content and length are the most reliable predictors of stability of dsDNA in any environment. DNA fragments containing lower percentages (<45%) of GC sequences are less stable as dsDNA and are more likely to be degraded into ssDNA³⁷⁻³⁸. Based on our data, dsDNA appears to also be preferentially adsorbed by several clay minerals, thereby increasing the amount of persistent DNA in the environment that competent bacteria may have access to. In addition, because maize DNA has an estimated GC content of 42.3%-55.4%²⁴ (with any variations occurring because of the number of redundant transposon sequences within the maize genome) it is possible that transgenic maize DNA would be more stable in the environment, allowing for added protection from DNase degradation and more opportunity to find and adsorb onto clay particles.

Conclusions

The acquisition of antibiotic resistance occurs naturally in the environment as a result of the horizontal gene transfer of ARGs. Selective pressures in the form of background concentrations of several antibiotics are known to be present in the environment as a result of antibiotic-producing fungi or the disposal of pharmaceuticals, which act as a selective pressure to induce the uptake, transfer, and maintenance of ARGs by sensitive bacterial communities³⁹⁻⁴⁰. However, there are several additional avenues through which bacterial communities may have gained access to ARGs and developed antibiotic resistance. Such avenues include the misuse of antibiotic prescriptions for illnesses, the

widespread use of concentrated animal feeding operations (CAFOs), and historical use of ARGs as selection markers in some lines of GM crops. Our results show that transgenic maize DNA is comparable to previous model DNA systems in terms of its adsorption capacity for clay minerals and soils. This adsorbed DNA could potentially persist in soils, especially in clay matrices commonly used in agriculture. It should be noted that we did not investigate the integrity of the maize DNA after the DNA extraction protocol to determine if its incorporation into a bacterial genome led to the acquisition of the antibiotic resistant phenotype. We also could not control for the size or GC composition of the extracted DNA fragments in our experiments. However, we determined through end-point PCR that some fragment of the *bla* ARG contained within the transgenic maize line we chose to investigate was present in the final DNA elution, which was then used to construct the adsorption isotherms. In conclusion, the overall adsorption capacities of montmorillonite and kaolinite appear to be significantly lower than the values cited in previous literature by one to two orders of magnitude. Despite these differences, the amount of transgenic maize DNA that can potentially adhere to soil matrices in the environment is still significant, approximately 70 µg of DNA per dry gram of soil. We would therefore conclude that the risk of microbial exposure to ARGs contained within transgenic maize lines, and any uptake thereof, should be investigated more thoroughly.

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