Presence of Emerging Contaminants and Antibiotic Resistance Genes in Private Well Water

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ABSTRACT:

Occurrence of emerging contaminants (ECs) including parent compounds, their metabolites, and antibiotic resistance genes (ARGs) and their relationships to common chemical and microbial indicators have rarely been investigated for private wells. In this study, occurrence of 109 parent compounds (ECs), 29 EC metabolites, and 15 ARGs were screened in private well water samples from 57 Southwest Virginia households and in municipal drinking water samples from similar areas. In the U.S., private well water is not regulated under the Safe Drinking Water Act like municipal water systems. Although well construction regulations exist in Virginia, there is no requirement for water testing, treatment, or system maintenance beyond the initial construction of the well. We found that 58 ECs, 18 EC metabolites, and 10 ARGs were detectable in at least one of the well water samples. Homeowner-reported household point of entry water treatment devices reduced the occurrence of ECs and EC metabolites, but some were not effective in eliminating the presence of ARGs. Oxcarbazepine, methylparaben, gabapentin, and triclosan were the most frequently detected in the well water in 68.4%, 66.7%, 61.4%, and 61.4% percent of samples, respectively. Ten ARGs were detectable in at least one well water sample, with *str*A (52.6%), *sul*2 (50.9%), and *tet*W (50.9%) being the most frequently detected. The overall number of detectable ECs, EC metabolites, and ARGs as well as levels of caffeine, tylosin, and triclosan in the well water were similar to municipal water samples. The occurrence of ECs, EC metabolites, and ARGs was not significantly different between the well water samples from the two counties, nor were they correlated with the homeowner-reported well depth, age, or distance from an onsite septic system. Occurrence of ECs and EC metabolites in the well water samples was positively correlated with some chemical water quality constituents (e.g., nitrite, sodium, and heavy metals), but not with total dissolved solids (TDS) or microbial-indicators (e.g. total

coliform bacteria, *E.coli* bacteria). However, microbial indicators were positively associated with the occurrence of ARGs. This study provides evidence of widespread occurrence of emerging contaminants in private well water samples and suggests the possibility of further ARG transfer among microbial constituents.

Keywords: emerging contaminants, antibiotic resistant gene, private well, occurrence

INTRODUCTION

Emerging contaminants (ECs) including pharmaceuticals and personal care products (PPCPs), illicit drugs, metabolites, as well as antibiotic resistance genes (ARGs) are receiving increased attention due to their potential impacts on human and environmental health (1-5). Pharmaceuticals and personal care products and their metabolites released into public sewers and private septic systems can eventually make their way into the environment (6, 7). Additionally, widespread use of antibiotics can result in elevated levels of ARGs in the environment (8). Once in the environment, these ECs may eventually reach groundwater, which is often used as a source for drinking water (9).

Numerous ECs have been detected in surface water, groundwater, treated and untreated wastewater, and municipal water supplies (10-15). Acetaminophen, carbamazepine, caffeine, cotinine, and metformin were the most frequently detected ECs in surface waters (13). These ECs were also detected at trace concentrations (i.e., ng L^{-1} to µg L^{-1}) in groundwater (16). It has been reported that the most commonly detected ECs in groundwater worldwide are caffeine, carbamazepine, diclofenac, ibuprofen, and sulfamethoxazole (17). To date, a limited number of studies have examined the occurrence of ECs in private well water supplies (18-20). No studies have attempted to investigate the co-occurrence of ECs and ARGs or the potential for common well water treatment devices to remove them.

In the U.S., there are about 23 million housing units (17% of the population) rely on private drinking water wells (< 25 people served or < 15 service connections for > 60 days/year) (21), none of which are not regulated by the U.S. Safe Water Drinking Act (22, 23). These private water supply systems are more likely to be in rural communities (24), more likely to be closer to potential pollution sources, and more likely lack the resources necessary for proper management

and maintenance of their water supply system (25). In Virginia, 1.7 million residents (approximately 20% of the population) rely on private well water supplies (26). To help ensure that the use of private wells does not negatively affect groundwater quality or public health, Virginia enacted regulations for the location and construction of groundwater wells (Virginia Department of Health, 1992). A Virginia Cooperative Extension program offered through Virginia Tech, the Virginia Household Water Quality Program (VAHWQP), helps homeowners who rely private water supplies test and learn about their water quality, how to maintain their private water supply systems, and how to address problems with source-water protection or treatment. The VAHWQP regularly offers county-based drinking water clinics in over 65 of Virginia's 95 counties each year through which participants submit homeowner-collected tap water samples that are analyzed for common chemical and biological water quality constituents. VAHWQP participants learn about well maintenance, groundwater protection, and ways to address water quality issues that may be identified (27). Since 2008, VAHWQP has analyzed over 24,000 samples serving over 50,000 individuals. The VAHWQP water quality database and participant pool, were leveraged to recruit voluntary participants for this study.

The objectives of this study were to: 1) determine the occurrence of multiple ECs, EC metabolites, and ARGs in private well water supplies in two southwest Virginia counties; 2) examine the relationships between common chemical- and microbial-water quality constituents and the occurrence of ECs and ARGs in the tested well water; and 3) evaluate the potential for common (homeowner-reported) private water supply treatment devices that may assist in the removal of ECs, EC metabolites, and ARGs from private well water supplies. To the best of our knowledge, no other prior studies have examined the co-occurrence of ECs and ARGs in private well water supplies in Virginia.

MATERIALS AND METHODS

Study sites and sample collection

Private well water samples were collected from 57 households located in two southwest Virginia counties (34 from County ID1 and 23 from County ID2) (S1, Supplemental Information). These two counties had relatively high numbers of previous VAHWQP clinic participants and provided a good opportunity for recruiting study participants. All study participants previously participated in a VAHWQP drinking water clinic. According to the 2020 US census (28), these counties have similar populations and demographic characteristics, although parts of County ID2 are more urbanized and densely developed than County ID1. An Institutional Review Board (IRB) protocol through Virginia Tech was in place to document how participants were contacted and recruited, how samples were collected, and how data was handled to protect the confidentiality of participants. The wells from the participating households were predominantly characterized as drilled wells constructed from 1950 to 2016 with depths ranging from 52 to 520 m (S1, Supplemental Information).

From each participating household, a 1.5 L water sample was collected by a trained graduate student directly from the kitchen tap after allowing the water to run freely for 1 min (20). A total of 57 water samples were collected. Out of the 57 participating households, 30 reported using various point of entry (POE) water treatment devices to treat the well water (Table S2 of Supplemental Information). For 11 of the households that self-reported the presence of POE water treatment devices, a separate1.5 L water sample was also collected from a bypass on the treatment device, from an access point upstream of (before) the treatment device, or from an outdoor spigot that was not connected to the treatment device. For these 11 households, the occurrence of ECs, EC metabolites, and ARGs in the untreated water (before POE(s)) was

compared to that for the corresponding treated kitchen tap water samples (after POE(s)) to demonstrate the potential for common (homeowner-reported) private water supply POE treatment devices to remove ECs, EC metabolites, and ARGs. The remaining 19 households who reported using of POE(s) lacked the access points that bypass the treatment device(s) and, therefore, water samples before POE treatment device(s) were not collected from these households.

During each onsite water sample collection, participants completed a survey. The survey instrument gathered information about homeowner's knowledge of well construction (e.g. depth, age), septic tank maintenance, and the use of personal care products, pharmaceuticals, pesticides, and various other chemicals by household inhabitants (Table S1, Supplemental Information). Table S2 (Supplemental Information) contains site and water supply system information provided by participants. Participant survey responses (e.g., age and depth of well, type of water treatment device) were not independently verified. In addition to the analysis for ECs, EC metabolites, and ARGs, each sample received a standard VAHWQP analysis of their water at no cost (value $55) as an incentive for participating in the research study. Table S3 describes the typical VAHWQP list of analytes that were also tested on the collected water samples. Any associations of these contaminants with the presence of ECs, EC metabolites, and ARGs were analyzed.

Five municipal water samples (MDW1 to MDW5) supplied by surface water were collected from the same two counties where the private well water samples were collected. A sixth groundwater-supplied municipal water sample (MDW6) was collected from a neighboring county (Table S4). All municipal water samples were collected using the same protocol as the private water samples. Because a point of use (POU) water treatment device (PUR Ultimate 11

Cup Pitcher with LED) was used at one of the households where a municipal water sample was collected, a treated water sample was also collected in addition to the tap water sample at this household (Table S4).

Glass bottles used for the sample collection were acid-washed and methanol-rinsed before collection. Water samples were stored in a cooler packed with ice and transported to Virginia Tech the same day. Samples analyzed for ECs, EC metabolites, and ARGs were stored in a -20°C freezer until analysis. Analyses for the VAHWQP analyte suite (Table S3) was performed according to VAHWQP protocols (Section S1 of Supplemental Information).

ECs and EC metabolites analysis

All water samples were pre-filtered through 0.7 μm glass fiber filters (Whatman, Maidstone, UK). Triplicate samples (200 mL each) were then each cleaned up of background interferring matrices and extracted for the target analytes using a solid phase extraction (SPE) method described in Section S1 of the Supplemental Information. The final extracts were then qualitatively screened for the presence of a total of 109 ECs and 29 EC metabolites (Table S5) on an ultra-performance liquid chromatography-tandem mass spectrometry (UPLC/MS/MS) using the method described in the Section S1 of the Supplemental Information. The non-target multi-compounds screening approach employing UPLC/MS/MS used a custom-made compound identification database and was semi-quantitative. This approach enables calculation of percent change based on peak areas of a screened compound in samples among those tested within one set and can help identify compounds that may be of interest for subsequent targeted quantitative testing by comparison with analytical standards.

Because quantification for all 138 compounds that were screened for in all water samples was prohibitively expensive and time consuming, three compounds; caffeine, triclosan, and

tylosin were chosen for further quantification in selected water samples on the UPLC/MS/MS (Section S1 of the Supplemental Information). These three compounds have been frequently reported to be present in various environmental water samples, and they were detected at higher frequency compared to other screened compounds in the samples collected for this study. The absolute levels of these three compounds were used to provide a perspective comparison among the well water samples, the municipal water samples, and various other environmental water samples reported in the literature.

ARG analysis

Total DNA was extracted from a 0.5 L water sample using the PowerWater DNA isolation kit (Mo Bio Lab., Carlsbad, CA) following the manufacturer's instructions. Fifteen ARGs conferring resistance to eight antibiotic classes were analyzed using polymerase chain reaction (Table S7). These fifteen ARGs have been frequently reported to be present in groundwater from various locations (29). All polymerase chain reaction (PCR) assays were conducted in a 20 μL volume reaction vial on an Eppendorf Mastercycler® ep Realplex real-time PCR system (Hamburg, Germany). The sequences of primers and annealing temperature of each targeted gene are listed in Table S7 in the Supplemental Information. The temperature program of PCR was as follows; initial denaturation at 95^{o}C for 10 min, followed by 40 cycles of 60 s at 95^{o}C, 60 s at different annealing temperatures, and 60 s at 72^{o}C (signal collection). Product specificity was visualized in agarose gels. Sterile water was used as a negative control in every run. PCR-grade water was used as laboratory blanks.

Statistical analysis

The detection frequencies of each of the 109 ECs and 29 EC metabolites and the 15 ARGs were calculated by dividing the number of samples in which a compound or ARG was

detectable by the total number of samples tested. Comparison of the concentrations of caffeine, triclosan, and tylosin in the well water and in the municipal water samples was done using the Kruskal-Wallis Test. Comparison of detection frequencies of ECs, EC metabolites, and ARGs between the well and the municipal water samples was accomplished using the Mosaic Plot and Chi-Square Test. The Kruskal-Wallis Test, Mosaic Plot, and Chi-Square Test were conducted using the JMP®, Version *14.0* (SAS Institute Inc., Cary, NC). For all statistical testes, a P value < 0.05 was considered significant. The correlation analysis between compound and ARG detection frequencies and common chemical- and microbial-indicators in the water samples was conducted using the Canonical correspondence analysis (CCA) in R with the vegan package (30).

3. Results and discussion:

Presence of ECs, EC metabolites, and ARGs in private well water

Compared to the municipal water samples, the well water samples contained higher numbers of ECs and EC metabolites, however, the occurrence of ARGs in both water sources (municipal and private well water) was similar. Out of the 109 ECs, 29 EC metabolites, and 15 ARGs screened for this investigation, 49 ECs, 13 EC metabolites, and 10 ARGs were detectable in at least one of the 57 well water samples collected from household kitchen taps. In comparison, 12 ECs, 3 EC metabolites, and 9 ARGs were detectable in at least one of the six municipal water samples.

Figure 1 lists the ECs with detection frequency higher than 5% of the 57 well water collected from the kitchen taps and 6 municipal water samples. Five anticonvulsants including oxcarbazepine, gabapentin, carbamazepine, pregabalin, and lamotrigine were detectable in 68, 58, 12, 12, and 7%, of the 57 well water samples collected from kitchen taps, respectively. While oxcarbazepine and carbamazepine were detectable in five and two of the six municipal water

203 samples, respectively (Fig. 1). Five antidepressant/antipsychotic ECs (paroxetine, O-
204 desmethylvenlafaxine, escitalopram, bupropion, and aripiprazole) were found in 25% of the well
205 water samples. Only one municipal water sample contained bupropion and aripiprazole.
206 Caffeine, a natural stimulant consumed by about 85% of the adult U.S. population daily (31),
207 was detectable in almost 50% of the well water samples and in all six municipal water samples.
208 Opioid pain relievers including buprenorphine, oxycodone, tramadol, and hydromorphone were
209 detectable in 5- to 23% of the well water samples, while buprenorphine was present in only two
210 of the municipal water samples. Acetaminophen, an over the counter pain reliever, was
211 detectable in 10 well water samples and was not present in any of the municipal water samples.
212 Five antibiotics including tylosin, sulfamethazine, trimethoprim, and sulfamethoxazole were
213 detectable in 9 to 25% of the 57 well water samples. Of these antibiotics, tylosin was detectable
214 in only one municipal water sample. Triclosan, a commonly used biocide in a wide range of
215 consumer products (e.g. soap, detergents, toothpastes), was detectable in 73% of the well water
216 samples and in five of the six municipal water samples. Thiabendazole, a commonly used
217 fungicide in pet care, was detectable in about 10% of the well water samples but not detected in
218 any municipal water samples. Other ECs including methylparaben (preservative), metformin
219 (diabetes medication), diphenhydramine (antihistamine), carisoprodol (muscle relaxer),
220 hydrochlorothiazide (hypertension medication), phentermine (diet drug), famotidine (acid reflux
221 and heartburn), lidocaine (topical anesthetic), and pseudoephedrine (decongestant) were
222 detectable in 5 to 60% of the tested well water samples. Metformin, a commonly prescribed
223 diabetic medication, appeared in all six municipal water samples. Although not detectable in the
224 well water samples, valsartan, a common prescription hypertension medication, was detectable in

three of the six municipal water samples. Both carisoprodol and phentermine were detectable in one municipal water sample.

Compared to the number of ECs detectable in the well and municipal water samples (Fig. 1), detectable EC metabolites were significantly less common (Fig. 2). Only four EC metabolites: cotinine (metabolite of nicotine), hydroxybupropion (metabolite of bupropion, an antidepressant), norsertraline (metabolite of sertraline, an antidepressant), and clopidogrel carboxylic acid (metabolite of clopidogrel, a blood thinner) were detectable in 5 to 10% of the 57 well water samples. In the six municipal water samples tested, cotinine was detected in four samples, carbamazepine-10,11-epoxide (metabolite of carbamazepine, an anticonvulsant and neuropathic pain medication) was detected in three samples, and N-desmethylcitalopram (metabolite of citalopram, an antidepressant) was present in two samples.

As shown in Fig. 3, a total of ten ARGs that are resistant to aminoglycoside, sulfonamide, tetracycline, macrolide, cephalosporin, and triclosan were detectable in 16-50% of the 57 well water samples collected from kitchen taps. About 50% of the tested well water samples contained strA (aminoglycoside-resistance gene), sul2 (sulfonamide-resistance gene), tetW (tetracycline-resistance gene), and ermB (macrolide-resistance gene). The same ARGs except the cephalosporin-resistant gene (ampC) were detectable in upto four of the six municipal water samples.

Because quantification of all 138 compounds was cost-prohibitive, three frequently detected ECs (caffeine, triclosan, and tylosin) consistently showing higher LC/MS/MS peak areas compared to the other compounds were selected for quantification in nine well water samples and one municipal water sample, to quantify concentrations of ECs in these samples (Fig. S1). Concentrations in the nine well water samples were: 0.69-1.5 µg/L for caffeine, 0.13-1.5 µg/L for

tylosin, and 0.78-54 µg/L for triclosan, while concentrations in the one municipal drinking water were 3.0 µg/L, 6.0 µg/L, and 23 µg/L µg/L, for caffeine, tylosin and triclosan, respectively. Previous investigations have reported slightly lower levels (0.10 to 0.23 µg/L) of caffeine in private and municipal wells of three other US states (32-34) and in US municipal water supplies (0.10 to 1.0 µg/L) (35). However, our reported levels are comparable to those previously reported for groundwater (16). Triclosan has been detected in about 8% of 73 US municipal water at concentrations less than the reporting limit of 1.0 µg/L (18). However, both triclosan and tylosin have not been documented previously in private well water.

Removal efficiency of homeowner-reported POEs

The presence of homeowner-reported POE water treatment systems seemed to reduce the numbers of detectable ECs and EC metabolites, but did not seem to impact the number of ARGs present in the POE-treated well water samples (Fig. 4). For the eleven well water samples that were treated by various homeowner-reported POE water treatment devices, 19 ECs had detection frequency of 5-63%, three EC metabolites had detection frequency of 10%, and 10 ARGs were detectable in 11-78% of the tested samples. The homeowner-reported types of POE water treatments included one or a combination of the following; UV (ultra-violet) light system, water softener, chlorination, sediment filter, carbon filter, iron removal filter, and acid neutralizer/calcite neutralizer (Table S2, Supplemental Information). Using a POE treatment device or a combination of POE treatment devices appeared to reduce the presence of ECs and EC metabolites in well water samples of 10 households but had no effect for the water in one household (site ID20) (Fig. 5a, Table S2). More variation was observed in the removal of ARGs in well water samples (Fig. 5b). For example, although usage of water softener was reported as the only POE treatment device used in households ID24 and ID28, the number of ARGs detected

in the water samples collected after this treatment device was higher than the number in the sample collected before this device (untreated water). However, a definitive conclusion cannot be drawn at this point because of lack of information on factors such as age and maintenance records.

In the one municipal water sample that was treated with a market-available POU (point-of-use) device (a water pitcher containing an activated carbon filter), eight ECs and eight ARGs were detected in the treated water sample, while four ECs and seven ARGs were detected in the untreated water (Figs. 1 & 3) but none of the EC metabolites were detected (Fig. 2). This filter might accumulate some ECs and harbor the development of ARGs during its lifetime, resulting in later release of ECs and ARGs into the treated drinking water. Although several studies have reported various removal efficiencies of different POUs for emerging contaminant compounds (36-38), to the best of our knowledge there is a lack of information on removal efficiencies of ARGs by POUs and how POU packing materials might affect ARGs. The test presented in this study was done for only one household POU and there was no knowledge of how this particular POU was used, for how long, and what the microbial community characteristics are on the packing materials. Further testing and characterization of more POU devices is needed.

Point of entry (POE) or point of use (POU) water treatment devices are often recommended as relatively low-cost remediation options for inadequate water treatment infrastructures in rural communities some of which solely rely on unregulated well water (39, 40). Nearly 40% of people in America use point of use (POU) water treatment devices at home to treat water before consumption. Many types of POE/POU water treatment devices available to consumers in the United States have undergone laboratory testing by the manufactures, but in most cases evaluations have focused on contaminants regulated under the Safe Drinking Water Act in the

United States (41). To date, there has been limited evaluation of the effectiveness of POE/POU water treatment devices for removal of non-regulated contaminants including ECs and ARGs from water. Recent research has shown that removal efficiencies of ECs by water treatment devices depend on the source water, brand of device, the volume of water treated, and routine care and maintenance of the devices (36). To verify which treatment devices are most effective in removing ECs and ARGs further research is required.

Well characteristics, common water quality constituents, and occurrence of ECs, EC metabolites, and ARGs in well water

The well characteristics reported by the 57 households are listed in Table S2. The 47 homeowner-reported well depths ranged from 16 to 259 m, with 50% of the wells deeper than 76 m, significantly deeper than the private wells (10 - 40 m) previously tested for ECs and reported in the literature (42, 43). Five respondents did not provide a well age/year of construction, but the homeowner-reported age of 52 wells that did supply this information ranged from 1950 to 2016. Half of the wells that were drilled prior to 1990 when the current Virginia Private Well Construction Act went into effect (44). All 57 households reported the use of onsite septic tanks, twelve of which were reported by participants as located less than the currently required setback of 30.48 m (100 ft) from the wellhead.

Overall, there was no correlation between the presence of ECs, EC metabolites, and ARGs with the homeowner-reported well depths and age (Fig. S2, Supplemental Information). Improperly constructed or maintained septic systems were proposed as a source of ECs in private wells (42). In this study (Fig. S3, Supplemental Information), although we observed slightly higher average numbers of detectable ECs and EC metabolites in the wells that were within 30.48 m of onsite septic systems compared to those that were further away, the difference was

317 statistically insignificant. Similarly, no statistical significance was observed between the
318 numbers of detectable ARGs in the water and the distance of the wellhead from the septic
319 system. We also did not observe a statistically significant difference between the occurrence of
320 ECs, EC metabolites, and ARGs in the wells sampled in two physiographic provinces (42 wells
321 were located in the Valley and Ridge province; and 15 wells were located in the Blue Ridge
322 province).

323 As shown in Fig. 6, the occurrence of detectable ECs and EC metabolites was positively
324 correlated with the concentrations of certain water chemical indicators, including sodium, nitrate,
325 and total heavy metals (sum of 16 heavy metals listed in Table S3 of SI). Positive correlation
326 between ECs and nitrate has been previously observed in urban riverine waters (45). The
327 presence of ECs and elevated nitrate in groundwater may be associated with anthropogenic
328 inputs. Correlations between the occurrence of ECs and nitrate, boron, and dissolved organic
329 compounds were considered as evidence that septic leakage was a possible source of ECs (42),
330 although in this study we did not observe a significant difference in occurrence of ECs and
331 ARGs in water from wells rgarudless of the self-reported distance to the septic system.

332 In this study, occurrence of detectable ECs and EC metabolites was not correlated with
333 the occurrence of ARGs, the presence of total coliform or *E.coli* bacteria, or total dissolved
334 solids (Fig. 6). This result is consistent with a study where a poor correlation between the
335 microbial indicators of fecal contamination and EC occurrence was observed (46). Our finding is
336 also consistent with another study where bacterial indicators did not correlate with elevated
337 concentrations of caffeine and nitrate (47). Our results indicate that the microbial- and chemcial-
338 indicator present in the sampled water may come from a different source or may have a different
339 fate reaching groundwater. The positive correlations between detectactable ARGs and the levels

of total coliform and total dissolved solids or even to certain extent the levels of *E.coli* bacteria (Fig. 6) suggest the possibility that those harmful pathogens might be a source of the detectable ARGs in the water and further ARG transfer among its microbial constituents.

Conclusion

Out of the 109 parent compounds (ECs), 29 EC metabolites, and 15 ARGs screened, 58 ECs, 18 EC metabolites, and 10 ARGs were detectable in at least one of the 57 private well water samples from two southwest Virginia counties. Concentrations of caffeine, tylosin, and triclosan (three ECs frequently reported in the literature) in nine well water samples were not statistically different to those in one municipal water sample. Homeowner-reported use of point of entry treatment (POE) devices seemed to reduce the occurrence of ECs and EC metabolites in treated well water samples, however, the effect of homeowner reported treatment devices on reducing the presence of ARGs in the water was inconclusive. Future research is needed to systematically investigate the removal effectiveness of specific types of POEs and the contributing factors for the removal. The occurrence of ECs, EC metabolites, and ARGs was not significantly different between the well water samples from the two counties that generally have differing under-lying geologies, nor was the occurrence of those same compounds correlated with the homeowner-reported well depth, well age, and well proximity to an onsite septic system. Positive correlations between well water chemical indicators and the occurrence of detectable ECs and EC metabolites, as well as positive correlations between the detectactable ARGs and the levels of total coliform, *E.coli* bacteria, and total dissolved solids in the well water may suggest their anthropogenic sources and the possibility of further ARG transfer among its microbial constituents.

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Figure Captions

Figure 1. ECs parent compounds with detection frequency of higher than 5% in the 57 southwest Virginia well water and six municipal water samples collected from kitchen taps. The asterisk indicates compounds that were detectable in the one municipal water sample that was treated with a pitcher-type filter, a point of use (POU) treatment device.

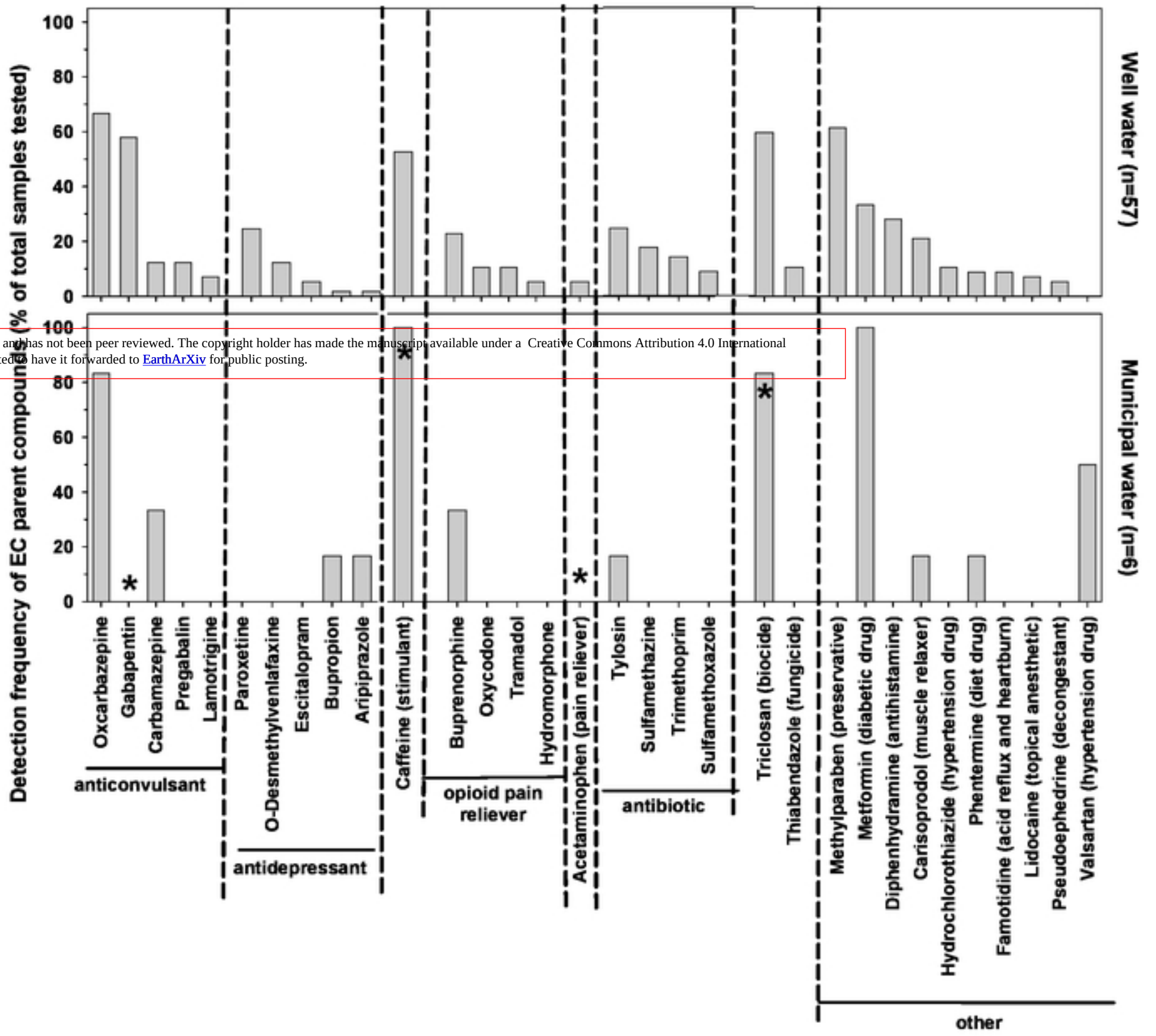
Figure 2. EC metabolites with detection frequency of higher than 5% in the 57 southwest Virginia well water samples and the six municipal water samples. None of the tested metabolites were detectable in the municipal water sample that was treated with a pitcher-type filter, a point of use (POU) treatment device. Parent compound (medical function noted in the parenthesis) of each detectable metabolite is listed on the 1st column of the table on the left.

Figure 3. ARGs with detection frequency of higher than 5% in the 52 southwest Virginia well water samples and the six municipal water samples. The asterisk indicates ARGs that were detectable in the municipal drinking water that was treated with a pitcher-type filter, a point of use (POU) treatment device. The antibiotic class for each detectable ARG is listed on the 1st column of the table on the left. #DNA quantity was too low in 5 well water samples to be tested for the target ARGs.

Figure 4. Detection frequency of: a) ECs, b) EC metabolites, and c) targeted ARGs in the 57 southwest Virginia well water samples collected before and after any homeowner-reported point of entry (POE) treatment device (Table S2, SI). #DNA quantity was too low in three POE-untreated and two POE-treated well water samples to be tested for the target ARGs.

Figure 5. Numbers of a) detectable ECs and EC metabolites; and b) ARGs in well water samples before and after treatment by the homeowner-reported POE treatment devices(s). *indicates no ARGs were detectable; # indicates insufficient DNA for testing the target ARGs. UV: UV light; WS: water softener; CL: chlorinator; SF: sediment filter; CF: carbon filter; IRF: iron removal filter; AN: acid neutralizer.

Figure 6. Canonical Correspondence Analysis (CCA) to elucidate the relationships between the concentration of tested water quality indicators (blue arrows), number of detectable ARGs (blue arrow), and the LC/MS/MS peak areas (yellow dots) of the detectable ECs and metabolites (yellow dots) in the 57 well water samples. Same arrow direction indicates a positive correlation, with longer arrow indicates a stronger relationship. Nitrate was reported as NO_3-N. Total heavy metals included the 16 heavy metals listed in Table S3 of SI.



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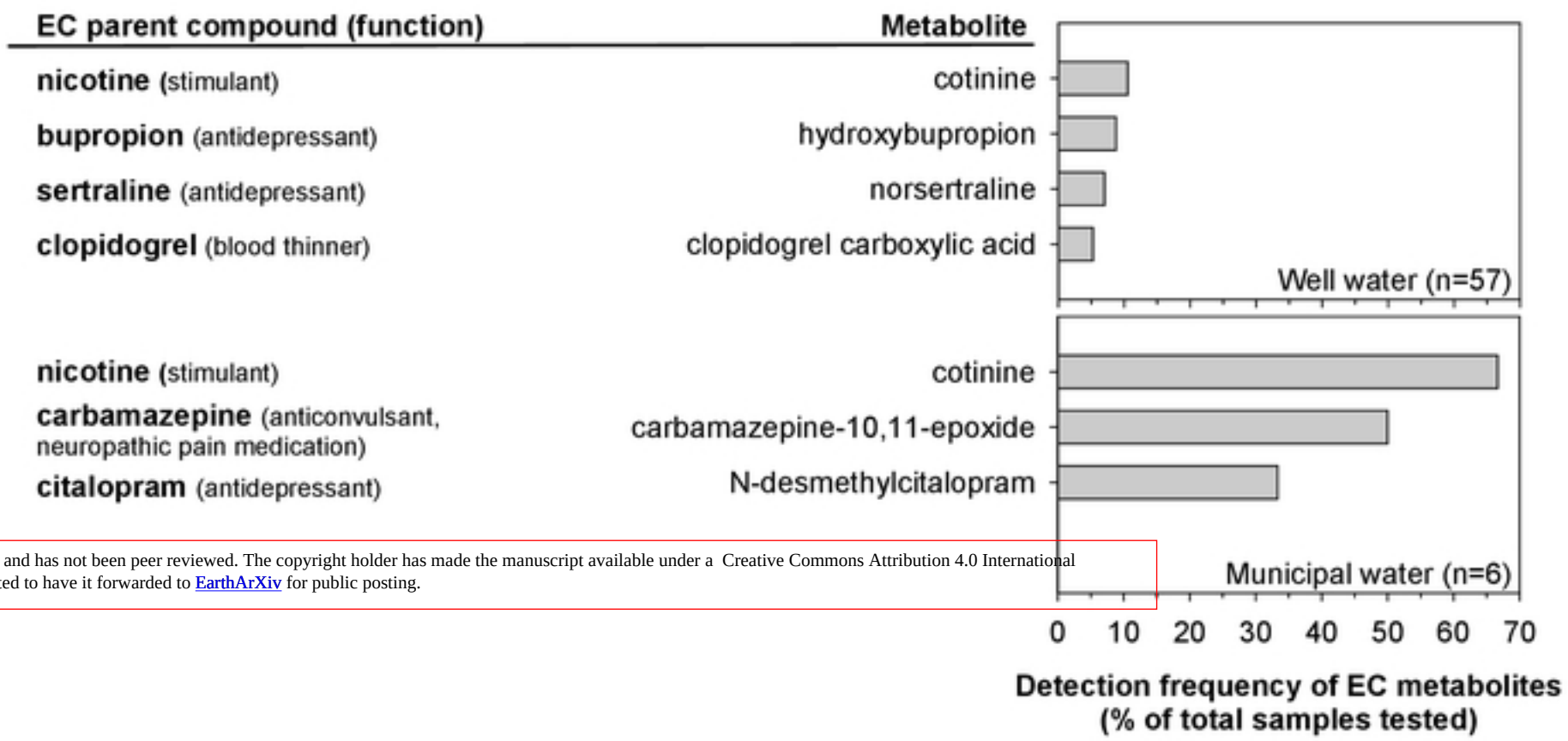

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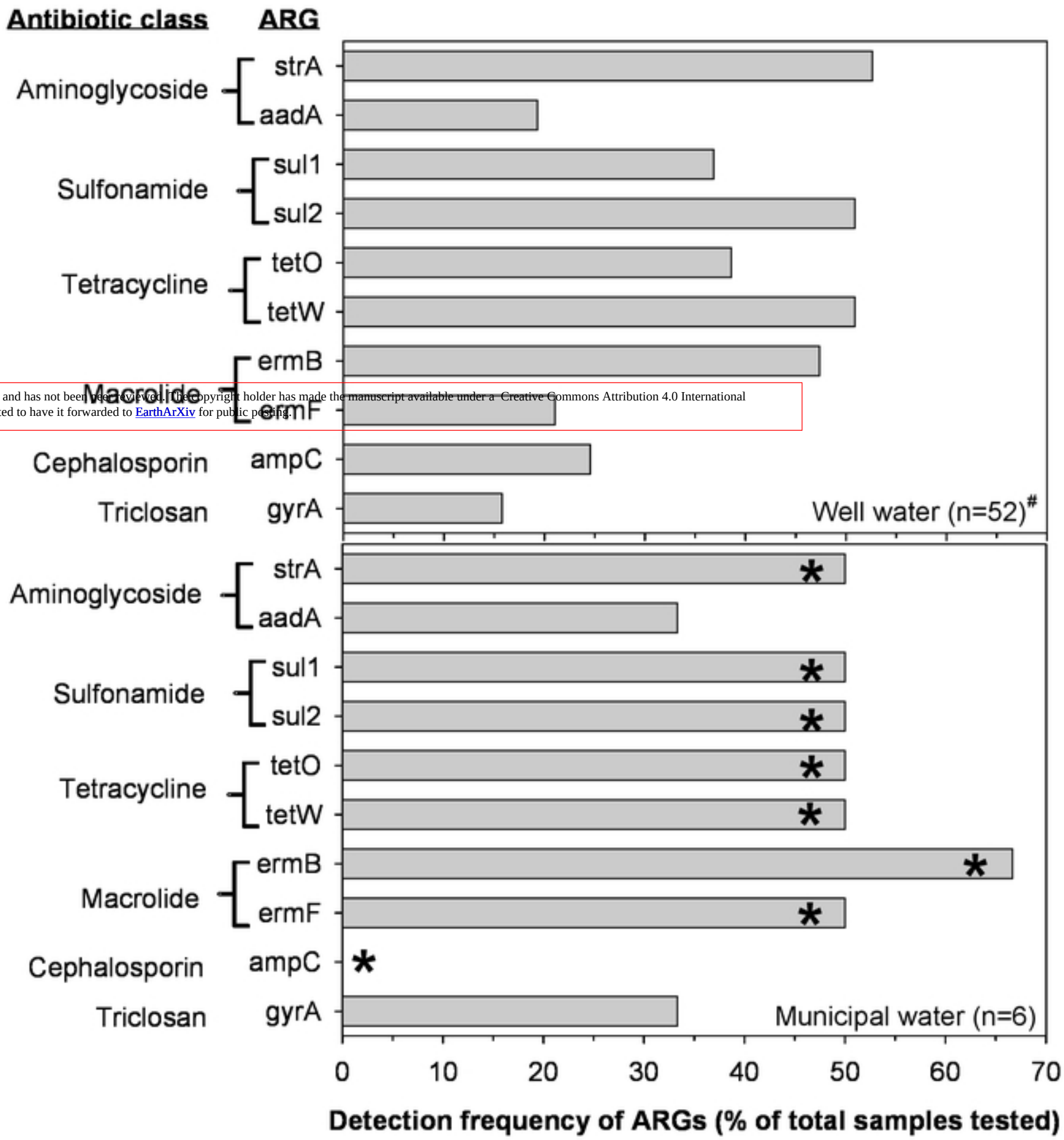

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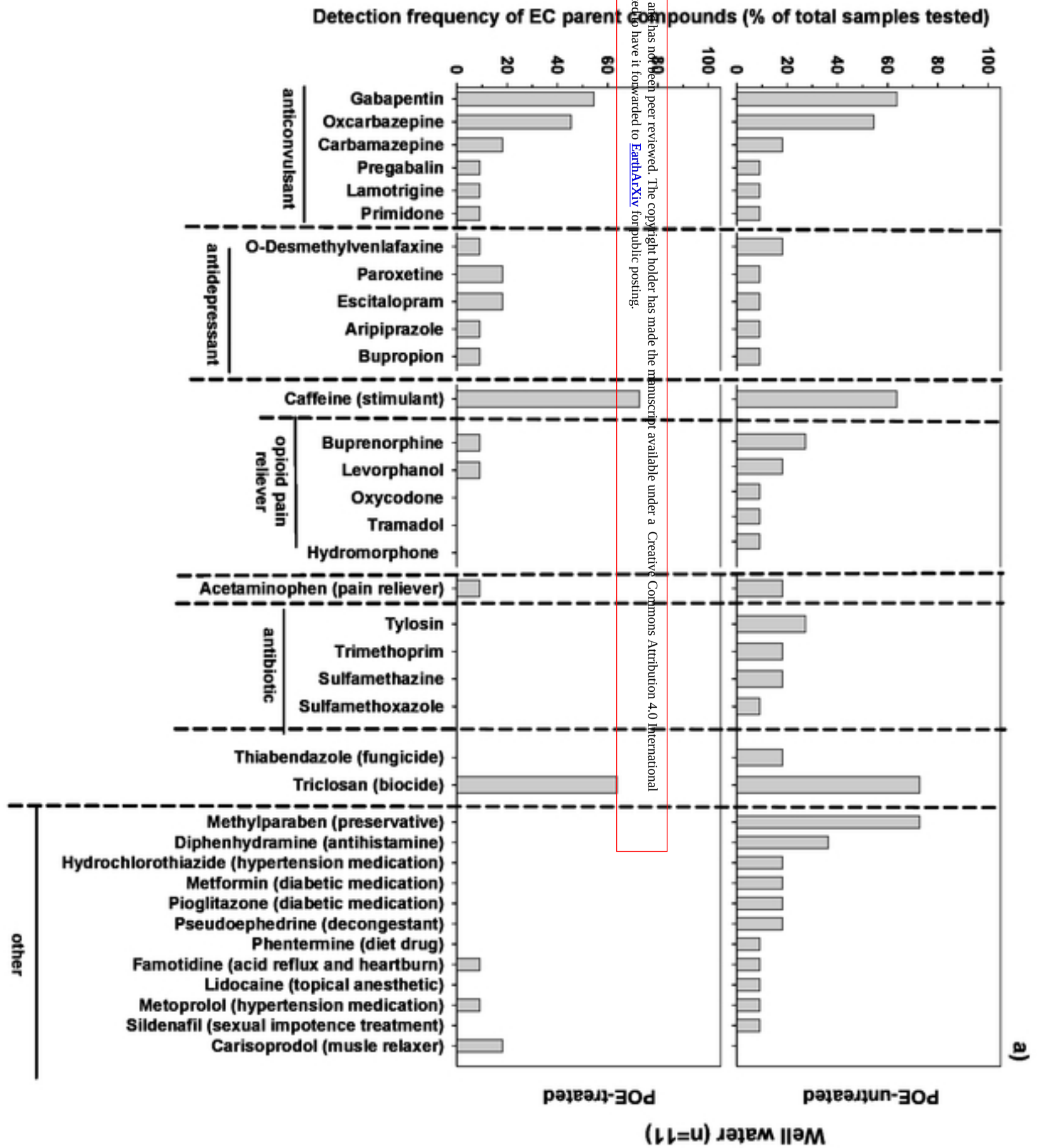

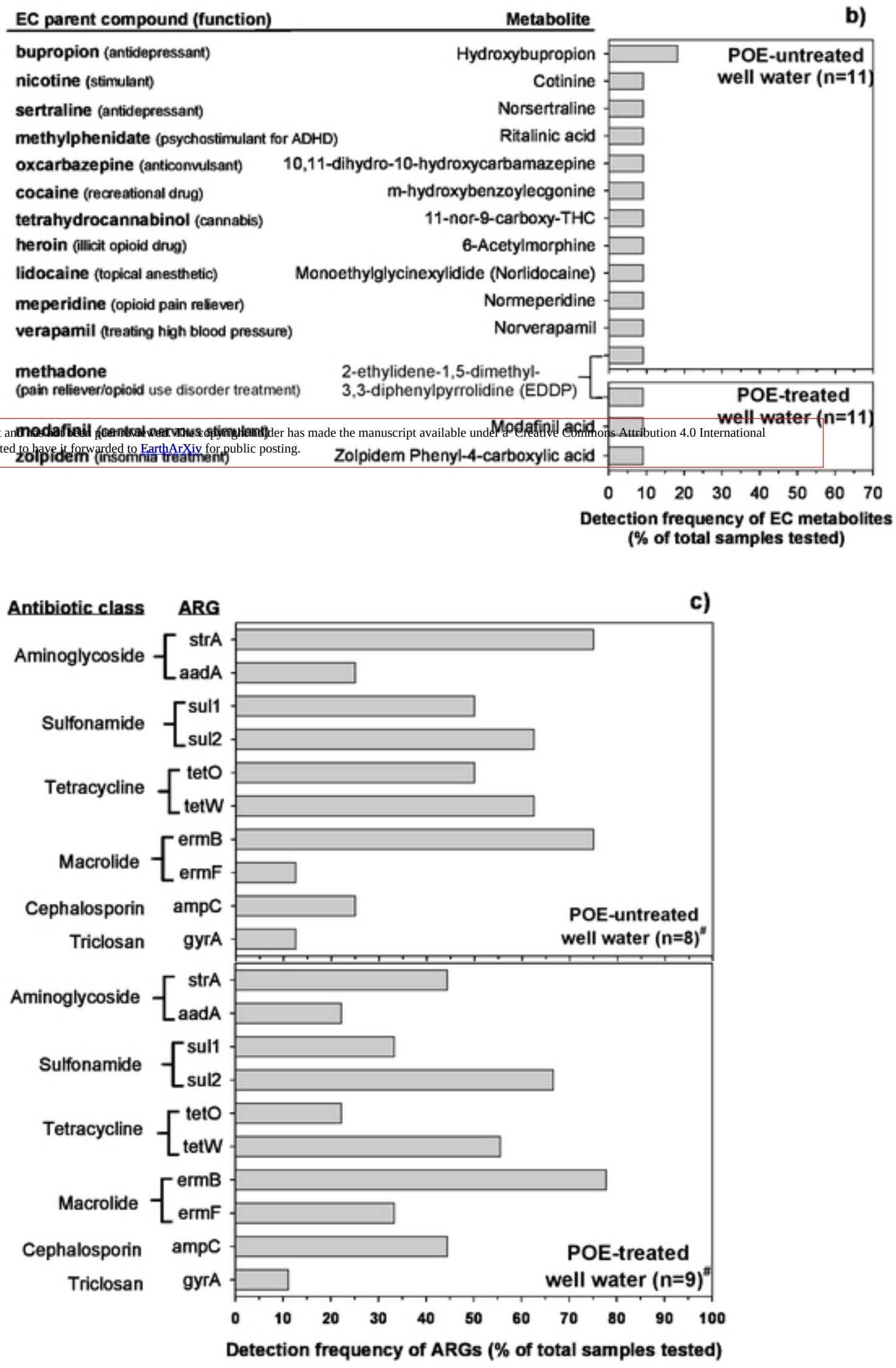

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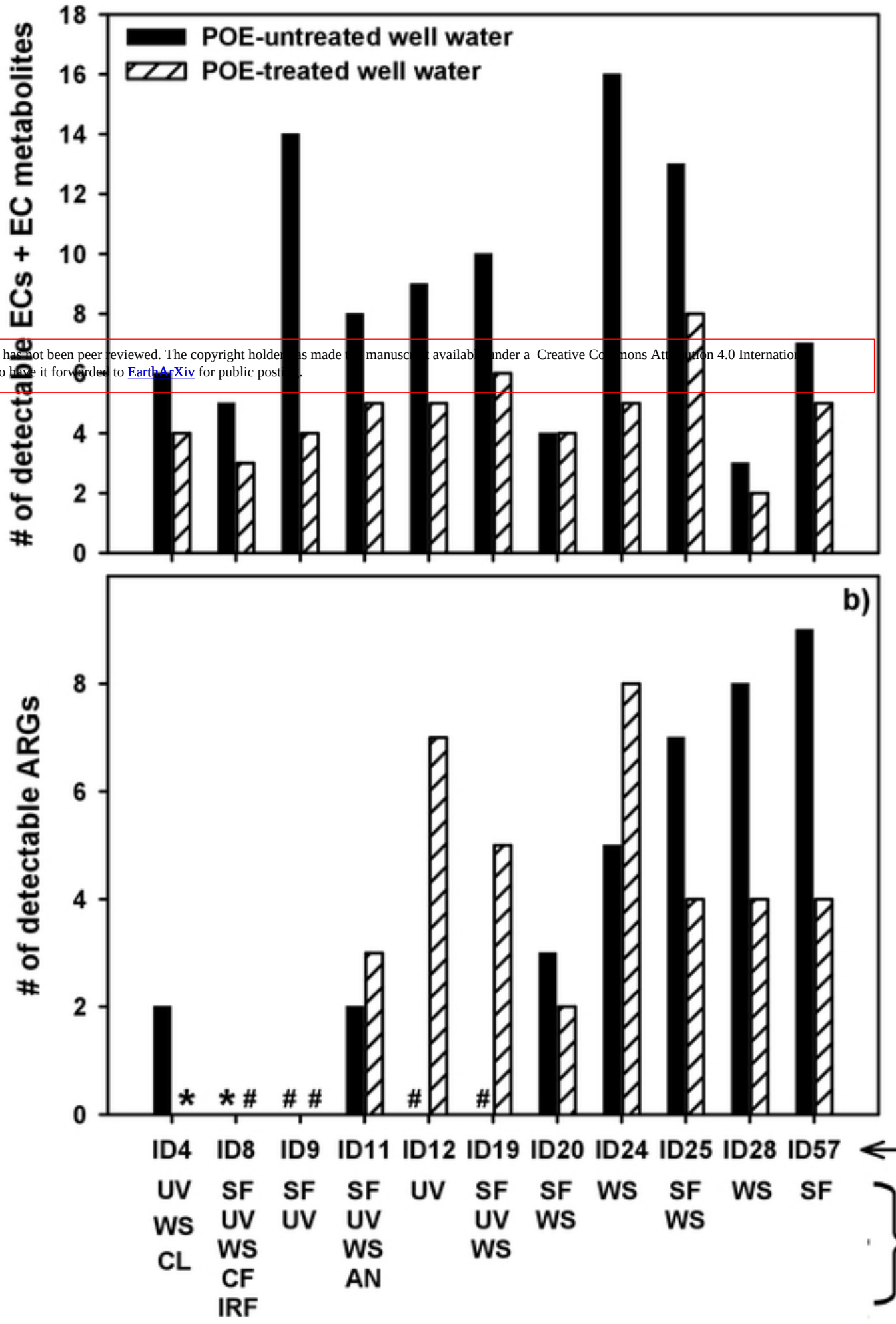

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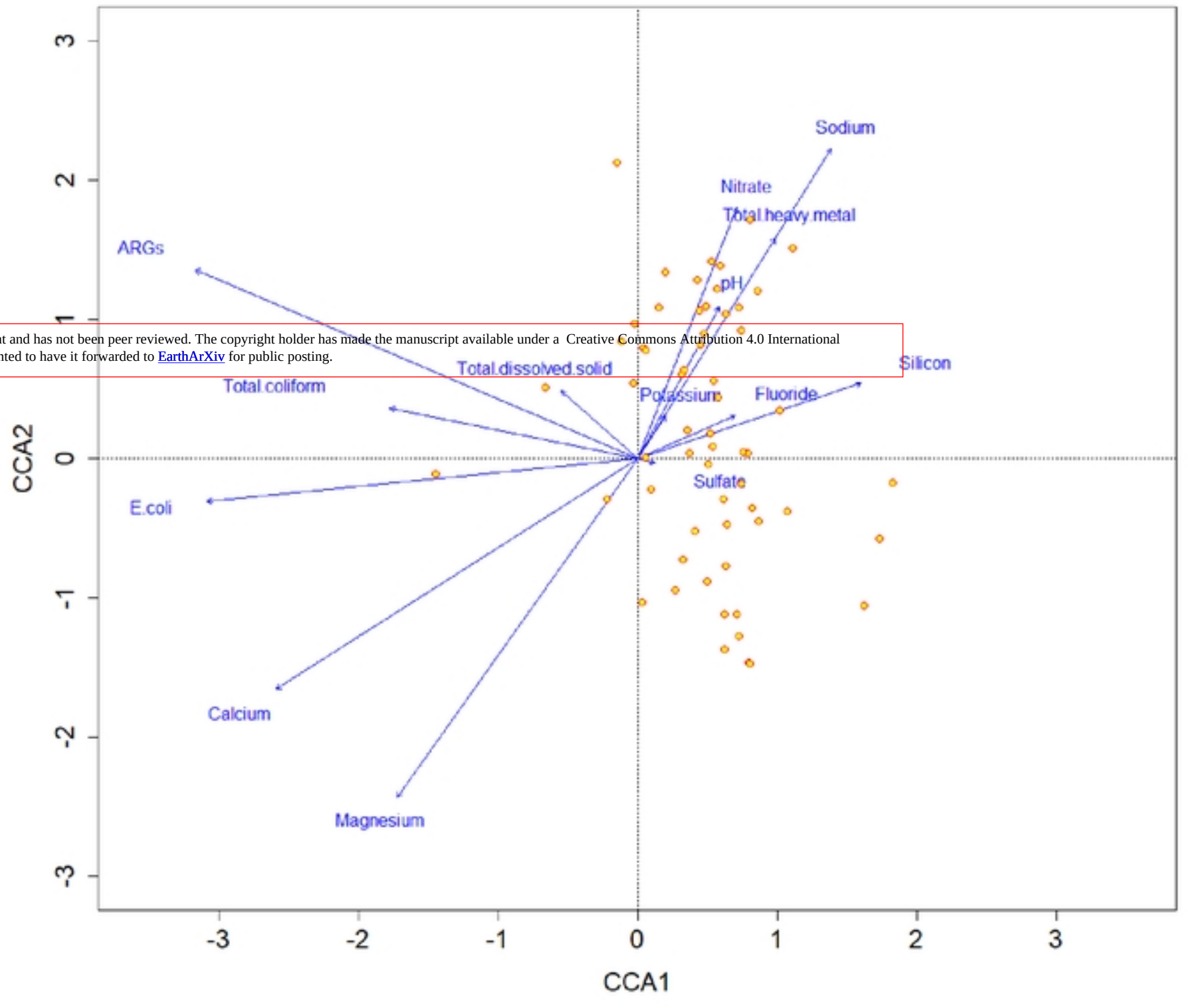

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