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Enhancing Confidence in Microplastic Spectral Identification via Conformal Prediction

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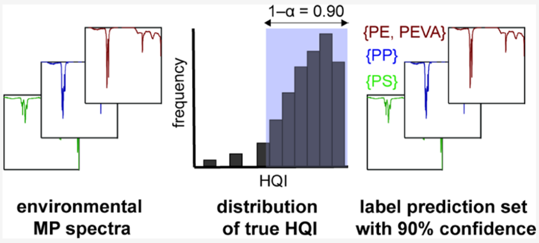

ABSTRACT: Microplastics are an emerging pollutant of concern, with environmental observations recorded across the world. Identifying the type of microplastic is challenging due to spectral similarities among the most common polymers, necessitating methods that can confidently distinguish plastic identities. In practice, a researcher chooses the reference vibrational spectrum that is most like the unknown spectrum, where the likeness between the two spectra is expressed numerically as the hit quality index (HQI). Despite the widespread use of HQI thresholds in the literature, acceptance of a spectral label often lacks any associated confidence. To address this gap, we apply a machine-learning framework called conformal prediction to output a set of possible labels that contain the true identity of the unknown spectrum with a user-defined probability (e.g., 90%). Microplastic reference libraries of environmentally aged and pristine polymeric materials, as well as unknown environmental plastic spectra, were employed to illustrate the benefits of this approach when used with two similarity metrics to compute HQI. We present an adaptable workflow using our open-access code to ensure spectral matching confidence for the microplastic community, reducing manual inspection of spectral matches and enhancing the robustness of quantification in the field.

KEYWORDS: *microplastics, conformal prediction, spectral matching, hit quality index, spectral libraries*

■ INTRODUCTION

Identifying microplastics (MPs) is essential to address health and environmental concerns caused by plastic production and pollution. Microplastics, defined as small pieces of plastic between 1 and 5000 μm,[1,2] have been found in environmental matrices such as seawater,[3−5] freshwater,[6−8] soil,[9,10] and air.[11,12] The extent of microplastic pollution and major MP sources, which are hypothesized based on the polymer identity of the MP,[13] must be characterized to develop remediation solutions and regulations. Researchers have employed techniques such as Fourier transform-infrared (FT-IR) and Raman spectroscopy to determine both the concentration and identities of MPs in the environment.[14−17]

Automated spectral matching has prevailed as a practical solution to time-intensive manual spectral matching of unknown environmental species to standard reference polymers.[18,19] Commercial software equipped with library-searching modules, such as Bruker's OPUS, Thermo Fisher's OMNIC, and Wiley's KnowItAll, have been employed to produce a similarity score between an unknown spectrum and a reference spectrum.[20−22] This score of spectral likeness, which is computed via similarity metrics, is referred to as hit quality index (HQI).[18,19] HQI scores are often reported in the range of 0 to 1, where a value of zero indicates the spectra are wholly unalike and a score of one indicates complete similarity.[18] Researchers commonly use a threshold HQI value that must be met or exceeded to identify unknown spectra with a polymer label.[18,19,23] However, there is disagreement regarding the meaning expressed by the HQI value, occasionally being referenced as a measure of confidence in a polymer label.[24−26] This assertion of confidence is misleading, as the HQI does not provide any measure of statistical certainty. Rather, the score simply reflects a measurement of the similarity between two spectra.

Because many polymers have similar structures (and therefore spectra),[27,28] and because vibrational spectra can change due to environmental aging,[29,30] labeling unknown spectra based on HQI scores alone is insufficient. A better approach for robust MP quantification and source attribution would include a measure of the statistical uncertainty in spectral matching. Acceptance of an identity solely due to an HQI score above an arbitrary threshold may lead to misidentification, as indicated by the findings of López-Rosales et al. in 2024, wherein a threshold of 0.85 (on a scale of 0 to 1) led to overestimating MP counts.[31] Indeed, voices in the MP community have called for stricter

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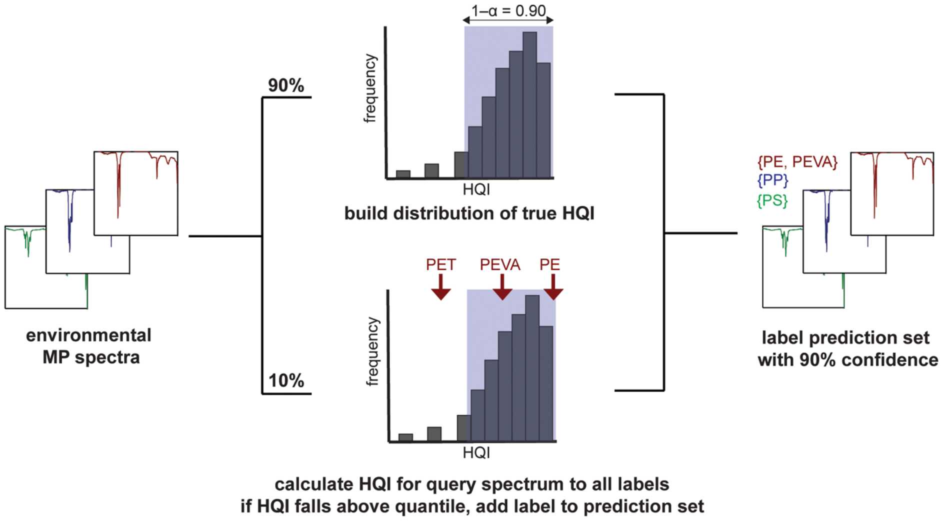
Scheme 1. CP Workflow, Where Exemplative Labels Corresponding to the Red Environmental MP Spectrum are Illustrated on the Bottom Distribution of HQI Scores from True Positive Matches



assurances of polymer identity through manual assessment of likeness between unknown spectra and standard references.[27,31]

To answer this call for increased confidence, some researchers have framed uncertainty in spectral labeling via distributions of errors between automated spectral match scores and expert manual assessments,[32,33] as well as through automated correlation scores of samples with known composition.[34,35] These approaches provide a guaranteed confidence for the whole sample, but for individual spectra, they are limited to accepting or rejecting a single spectral label. Moreover, these methods are asymptotically valid, meaning that the guarantees hold only when the number of observations (here, spectra) nears infinity. In contrast to previous methods, a method known as conformal prediction (CP) presents the advantages of indicating the uncertainty of individual matches and providing a theoretical confidence (or probability) guarantee that the identity of the query spectrum is contained in a set of predicted labels.

Conformal prediction is a framework that provides a principled approach to understanding uncertainty via model-generated predictions.[36,37] This method generates a prediction set of reference labels that guarantees a specified level of finite sample confidence,[38] meaning the probability that the correct spectral label is included in the prediction set of an unknown spectrum is equivalent to the confidence guarantee (given that the unknown's identity is reflected in the reference library). CP has been increasingly popular in machine learning, where it is able to provide insight into the uncertainty of a given model without the need for retraining, which is often computationally intensive.[39−41] Our introduction of CP to the MP community provides a tool for enhanced credibility in spectral matching via a statistical assurance that the identity of an unknown spectrum will be returned to the analyst.

Herein, we first evaluate the spectral correlation software, methods, and thresholds reported in the recent microplastic literature to establish a baseline of commonly used automated matching practices in the MP community. We then illustrate that the HQI scores generated by popular commercial software lack the clarity needed to confidently count and label environmental MPs. Further, we demonstrate the advantage of using CP in tandem with two different similarity metrics to provide a confidence guarantee and a measurement of uncertainty at the individual spectrum level. Lastly, we apply this method to authentic, environmental plastics to illustrate CP's utility with real-world samples. Through this work, we aim to provide a workflow to incorporate statistical assurances in microplastic spectral matching and establish guidance for HQI thresholding and label acceptance for more robust environmental MP identification.

■ METHODS

Literature Review. To illustrate the microplastic community's recent use of library searching software, metrics, and thresholds, we conducted a Web of Science search for articles published within the last eight months at the time of searching (May 2023 to January 2024) that utilized vibrational spectroscopy to identify MPs (see Supporting Information section I). To limit entries, we specifically targeted papers involving MP identification in an oceanic matrix; as this matrix has received the greatest attention of all environmental sectors, we expect that the methods used in these articles will best reflect the current state of microplastic spectral matching.[42,43]

Commercial Software Library Searching. The open-access spectral databases "FT-IR library of plastic particles" (FLOPP, used as a pristine reference library) and "FT-IR library of plastic particles sourced from the environment" (FLOPP-e, used as environmental query spectra) were chosen to evaluate commercial software due to their accessibility and widespread use in the microplastic community.[44−47] All individual spectra belonging to the polyethylene (PE) and polypropylene (PP) labels available in the FLOPP-e library were downloaded as .csv files and uploaded to Wiley's KnowItAll SearchIt function. These polymers were chosen because they had the greatest number of example spectra in the environmentally aged FLOPP-e library (49 and 66 for PE and PP, respectively). For each spectrum, a transmission mode was indicated, and a single component search was performed using the default settings of a correlation method and optimized corrections.[48] A spectral inclusion window was drawn from 4000 to 650 cm^{-1} to include only regions of the spectra where the data had been collected. Each spectrum was searched for correlation with pristine polymers in the FLOPP library, and the hit list was documented. The highest HQI score and label, as well as the number of matches and number of polymer labels yielding HQI ≥ 0.7 (a common literature threshold of HQI),[24,25,29] were recorded.

Conformal Prediction. Conformal prediction is used to generate prediction sets (of polymer labels) for new observations given a trained prediction model (HQI-based spectral matching). CP uses a calibration data set and similarity metric to ensure that the spectral label of a query spectrum will be returned in a prediction set with a user-specified probability (e.g., 90%), affording the confidence guarantee (see Supporting Information section III for information on the mathematical basis of the method). This confidence guarantee holds when the distribution of new observations (here, HQI scores) is identical to those in the calibration set. Unlike many other uncertainty quantification methods in the literature, CP does not require specific distributional assumptions (e.g., Gaussianity) nor does it require a large data set.

Using all spectra from the 11 labels (acrylonitrile butadiene styrene, cotton [C], polyamide, polycarbonate, PE, polyethylene terephthalate [PET], polyethylene vinyl acetate [PEVA], PP, polystyrene [PS], polyurethane, and polyvinyl chloride [PVC]) that appear in both the FLOPP and FLOPP-e data sets (142 and 189 spectra, respectively), we split the FLOPP-e spectra into calibration (90%) and test (10%) sets for matching to FLOPP reference spectra (Scheme 1). We then calculated the HQI scores of the true positive matches (FLOPP-e spectrum matching to an FLOPP spectrum of the same polymer label) of the calibration set and tabulated them in frequency distributions relating to the two different similarity metrics. Next, the remaining 10% of FLOPP-e spectra was used as a test set (meaning that they were treated as spectra of unknown identity) and the HQI scores for each spectrum's similarity to all reference polymers in the FLOPP library were calculated. These HQI values were compared to the threshold defined by the corresponding quantile of the calibration set distribution that resembles the guaranteed confidence (here, 90% [$\alpha = 0.1$]) for both similarity metrics. If the HQI of a polymer label was above the threshold, meaning that only 10% of the calibration data's true positive scores were calculated to be less than the HQI, then the label was added to the prediction set with an associated 90% confidence. In Scheme 1, this workflow is illustrated using example labels and HQI scores for the red FLOPP-e spectrum, which are then compared to the calibration distribution and added to a prediction set of labels (on the far right of the graphic) if the scores are greater than the established quantile.

The FLOPP-e spectra were randomly split into calibration and test data sets 500 times in a cross-validation fashion. We then calculated the empirical confidence (proportion of instances where the correct label was observed in the prediction set) and prediction set size (number of labels included in the prediction sets) for all of the test data.

When using CP, the analyst chooses the confidence guarantee that will determine the HQI threshold. While the confidence is always guaranteed given that the reference library contains spectra of each unknown's identity, the magnitude of the confidence guarantee influences the number of labels returned in the prediction set.[37] With a high confidence guarantee, the HQI quantile (i.e., threshold) is lower, leading to more polymer labels in the prediction set. On the other hand, a low guaranteed confidence may lead to very few, if any, labels in the prediction set. While this trade-off between confidence and prediction set size should be noted as inherent to the conformal prediction workflow, we provide insight into tailoring the confidence guarantee and leveraging the performance of the chosen similarity metric to produce the most insightful predictions in future sections.

Class-Conditional Conformal Prediction. One limitation of the traditional CP framework is that the confidence guarantee is marginal, meaning that some labels may be under or overguaranteed, as only the average theoretical confidence across all labels is assured.[39] To address this issue, we also employ the class-conditional conformal prediction (CC−CP) approach.[49] The basis of the CP methodology framework (i.e., create a calibration set, determine the HQI threshold, compare test set HQI scores to calibration distribution, and add labels with HQI scores represented above the quantile to the prediction set) remains the same. The only difference is that instead of constructing one distribution from the calibration set, we created separate distributions for each label to ensure class-balanced confidence and that the HQI scores per polymer type were compared to label-specific distributions (Scheme S2).

Because a unique distribution is built for each label, we used the five labels with 10 or more example spectra in the FLOPP data set (i.e., C, PE, PET, PP, and PS) as possible spectral labels, limiting the pool of FLOPP and FLOPP-e spectra to 76 and 161 examples, respectively. The FLOPP-e calibration and test data were then randomly split 500 times in a cross-validated fashion. Although the traditional CP results using these five labels are presented herein for direct comparison to the class-conditional results, it should be noted that additional labels can be used with a CP framework and that this addition of labels will result in larger average prediction sets (see Supporting Information section V).

Similarity Metrics Used to Compute Hit Quality Index. In CP, the choice of the similarity metric used to compute the HQI is crucial because while the confidence guarantee holds for any similarity metric, the quality of the similarity metric directly governs the size of the prediction set. The size of, or number of labels in, the prediction set can be used to assess uncertainty in the predictions,[39] where prediction sets with few labels indicate a low uncertainty and prediction sets with many labels indicate a high uncertainty. Efficient similarity metrics give prediction sets with fewer labels, therefore providing a more accurate depiction of the inherent spectral uncertainty in the prediction sets.[50] Thus, exploring different similarity metrics, which rely on different measures of spectral likeness,[18] is necessary to compare the uncertainty of resultant prediction sets. This aspect of CP led us to investigate nearest neighbor (NN),[50] and the more commonly used Pearson correlation coefficient (PCC), as similarity metrics to calculate HQI (see Supporting Information section II).

Application of Conformal Prediction to Real World Environmental Plastics. Environmental plastics of unknown identity were sourced from a residential parking lot in Brighton, Michigan and rinsed with tap water to remove debris (see Supporting Information section IV). After the plastic was dried at room temperature, attenuated total reflection FT-IR measurements of each plastic were collected in percent transmission using an Agilent Cary 630 FT-IR spectrometer. Background spectra were collected prior to sample analysis, and the diamond crystal was cleaned with ethanol between samples. To best mimic the FLOPP-e library (treated as the calibration set), the same spectral collection parameters of 32 coadded scans at 4 cm^{-1} resolution were used to collect signal between 4000 and 650 cm^{-1}. The unidentified spectra were manually evaluated by one researcher to label each spectrum with a primary polymeric identity based on labeled spectra in the

FLOPP and FLOPP-e libraries (see Supporting Information, section IV). Prior to accessing the manually identified labels, the CP method was applied to the environmental spectra (treated as the test set) by a different researcher. The spectral labels determined by manual evaluation were then used to calculate empirical confidence once the CP results were obtained.

RESULTS AND DISCUSSION

Literature Review. *Similarity Metrics Used to Label Unknown Spectra are Not Reported in the Literature.* We reviewed 96 recently published articles using vibrational spectroscopy to identify environmental MPs (see Supporting Information, section I). Database matching was the major routine identified (64%), followed by manual evaluation (8%), and machine-learning techniques (4%) (Figure S1). Alarmingly, approximately 24% of the published articles did not indicate how a spectral match was determined. Of the articles that indicated spectral database searching as the identification method, 74% did not report the similarity metric used to determine the match, which is concerning given that the algorithm chosen to analyze spectral similarity has a significant impact on the polymer label.[18,32,51] Approximately a quarter of the articles reported the similarity metric used to calculate HQI, with 22% using PCC (Figure S1). Our 2024 results closely mimic the findings of Weisser et al. in 2022, where the proportion of articles that failed to detail the data analysis routine and similarity metric used for database matching is the same (see Supporting Information section I).[18] It is concerning that there has been no observed response to their call for transparent communication of spectral identification methodologies.[18] The constant proportion of researchers who have omitted critical information between these two evaluations continues to jeopardize the robustness and validity of published MP identification findings.

There is No Literature Consensus on Software Used for Spectral Matching nor Threshold of HQI to Distinguish a Microplastic. Software applications specific to the parent company of the analysis instrument were reported to perform automated spectral matching in 51% of publications (Figure 1a). KnowItAll, a commercial software for spectral matching from Wiley BioRad, was used in 19% of publications, and open access searching platforms such as PlaMAPP,[51] OpenSpecy,[52] and siMPle[53] were used in 24% of publications. The default similarity metrics and preprocessing pipeline differ between the reported software, which obscures a direct comparison between HQI scores generated from different applications.[32,51,54] Despite published approaches on establishing consistent HQI thresholds,[32−35] neither a universal HQI threshold nor thresholding strategy was observed in the literature (Figure 1b). While a threshold of 0.7 was reported by 42% of considered publications, the explanation for this choice, when reported, was precedent, likely because the seminal microplastic paper published by Thompson et al. in 2004 used this value of similarity to accept plastic identification.[23] Approximately 31% of articles reported a higher threshold than 0.7, and about 20% of authors chose thresholds below 0.7. Considering the ambiguity and diversity of software and thresholds used to aid spectral matching, it is clear that the MP community would benefit from a method to directly compare the performance of similarity metrics and guidance to determine the HQI threshold with an associated confidence.

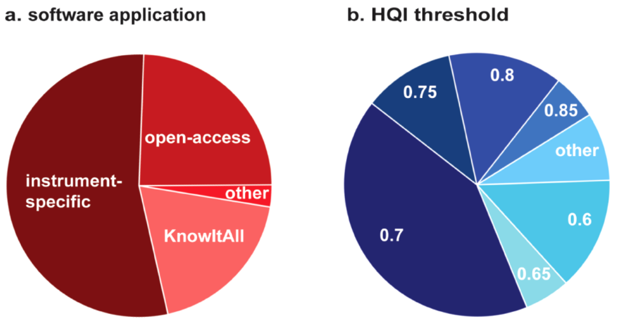


Figure 1. Summary of published MP papers regarding a) software applications used for automated spectral matching and b) HQI threshold used to accept a match.

Commercial Software Library Searching. *Similarity Metrics Alone Do Not Guarantee Confidence in a Spectral Match.* We searched all polyethylene and polypropylene FLOPP-e spectra as a substitute for unknown environmental spectra using the FLOPP reference library in KnowItAll. The reference spectrum identity with the greatest HQI was recorded as the label for the unknown spectrum, as is typical for MP identification workflows.[18,19] Overall, the 49 PE and 66 PP spectra from the FLOPP-e library were matched to four unique polymer reference labels (e.g., PE was matched with PE and three other polymers) with the topmost HQI (Figure 2a). Thus, if the traditional approach of accepting the label of the reference spectra with the greatest HQI was followed, numerous spectra would be mislabeled. This result was similarly reported by Primpke and co-workers in 2017, where the authors found that OPUS software misassigned spectral labels to query spectra using various data analysis routines.[32] When expanding the criteria of a spectral match to include any label returned with HQI ≥ 0.7, the matches to individual PE and PP spectra returned 7 and 11 unique labels, respectively (Figure 2a). It should be noted here that, as the FLOPP reference database contained 14 possible labels, 50% of available labels were assigned to PE spectra and 79% to PP spectra. Thus, an HQI threshold is not accompanied by any measure of certainty, as multiple reference spectra labels were matched to a single query spectrum with similar values of likeness. If we were to theoretically treat the HQI threshold as a measure of confidence in a match, then that confidence would not be exclusive to the single classification with the greatest HQI; rather, it would include all labels above the chosen threshold. Therefore, the results of this experiment, which replicated libraries and software similarity methods used in the current MP literature, illustrate that the identification output by these methods lacks any

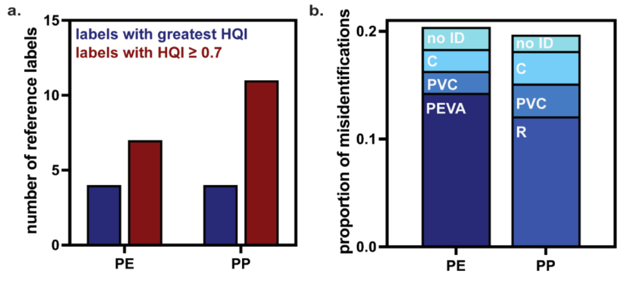


Figure 2. Results from KnowItAll searches of PE and PP FLOPP-e spectra in terms of a) number of reference labels returned and b) proportion of misidentifications when the greatest HQI ≥ 0.7 is accepted as a label, where "no ID" is unidentified and "R" is rubber.

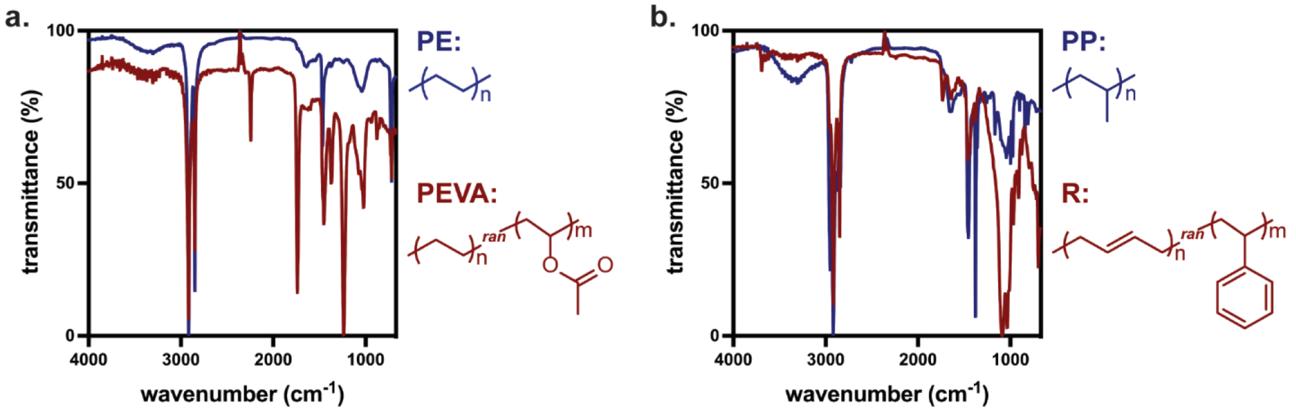


Figure 3. Average min−max normalized overlaid spectra and chemical structures of a) PE (49 FLOPP-e spectra) and PEVA (10 FLOPP spectra) and b) PP (66 FLOPP-e spectra) and rubber (R) (15 FLOPP spectra).

associated certainty. In fact, a top match will always be generated for a query spectrum, regardless of whether a spectrum of the same identity is even in the reference database.[55]

KnowItAll Misidentifies ∼20% of PE and PP Spectra, Largely Due to Chemical and Spectral Similarity Between Polymer Identities. We recorded the proportion of spectral misidentifications when the reference spectrum identity with the greatest HQI (above a 0.7 threshold) is accepted as a label, noting that ∼20% of PE and PP spectra within FLOPP-e were misidentified (Figure 2b). Individual spectra within the FLOPP-e or FLOPP library may suffer from a low signal-to-noise ratio or spectral artifacts known to decrease the accuracy of a matching metric,[19,54] which could account for instances of misidentification or instances where no labels were returned above the threshold (indicated as "no ID" in Figure 2b). However, we noticed that the greatest proportion of misidentifications seemed to have been caused by chemical, and thus spectral, likeness between the query and returned spectra. For instance, 70% of PE misidentifications were labeled as polyethylene vinyl acetate, which features the same hydrocarbon repeat unit as PE (Figure 3a). The main spectral distinction between these two species is due to the copolymerized vinyl acetate unit in PEVA, which exhibits distinct peaks around 1725 cm^{-1} (C═O stretch) and 1228 cm^{-1} (C−O stretch).[56] Similarly, 62% of PP misidentifications were labeled as rubber. The chemical structures of PP and styrene−butadiene rubber likewise share chemical functionalities (Figure 3b). For instance, styrene−butadiene rubber FT-IR spectra exhibit methylene bending and stretching vibrations similar to those observed in PP spectra.[57]

Current Approaches to Reducing Misidentification of Microplastics are Tedious and Lack Any Measure of Associated Confidence at the Single-Spectrum Level. Additional occasions of spectral likeness causing challenges in MP identification have been noted in the work of Schram and coworkers, where highly weathered PP was misidentified as chitin, and both PP and chitin were returned as labels with HQI greater than 0.7 for a less weathered PP sample.[29] Further, the spectral differences of some polyamides and biological materials such as skin cells and proteins are so slight that these materials are likely to be mislabeled when using traditional similarity metrics to output and accept a topmost HQI value.[54,58] The prevailing solution to this issue is to include both natural and synthetic materials in the reference library and to manually evaluate the matched spectra with HQI values near the greatest returned similarity value.[51,59,60] Unfortunately, this proposed solution does not impart any guidance on how many labels, or what lower limit of HQI, should be further evaluated, nor does it give the user any sense of confidence in the chosen label. On the other hand, an analytical workflow involving conformal prediction would establish a HQI threshold with guaranteed confidence and provide a prediction set of reference labels for streamlined manual inspection. When multiple labels are returned in a prediction set, those including synthetic spectral labels can be prioritized for visual evaluation against spectra of other natural or synthetic identities in the prediction set, and those containing only natural identities can be excluded from additional analysis. Because the confidence guarantee applies only to the prediction sets of spectral labels (rather than a summarized report of MP quantities or identities), visual evaluation of spectral similarity remains integral to environmental MP identification. Conformal prediction reduces this manual inspection and enables efficient spectral labeling with a confidence guarantee at the single-spectrum level.

Conformal Prediction. *Nearest Neighbor Produces Smaller Mean Prediction Set Sizes than Pearson Correlation Coefficient.* Using the five reference labels with 10 or more spectra in the FLOPP and FLOPP-e libraries, we quantified the mean empirical confidence and prediction set size of using CP with PCC and NN similarity metrics (Figure 4). As expected, the empirical confidence closely aligns with the confidence guarantee (90%) for both metrics. However, we observed a

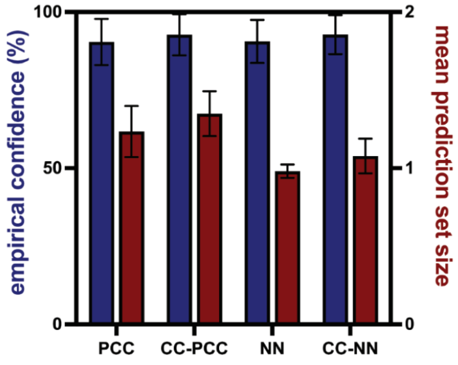


Figure 4. Empirical confidence and mean prediction set size results of conformal prediction using the Pearson correlation coefficient (PCC) and nearest neighbor (NN) and class-conditional conformal prediction. Here, CC-PCC and CC-NN correspond to the use of class-conditional conformal prediction with PCC and NN, respectively.

lower average prediction set size using the NN metric compared to PCC. The fact that PCC is an absolute metric whereas NN is a relative metric may contribute to this difference in the number of reference labels in the prediction set, as the criteria for generating a high HQI with NN are more restrictive than with PCC (see Supporting Information section II). A further explanation for this difference in metric efficiency relates to the distributions of the positive matches (i.e., correctly identified) and negative matches (i.e., mismatch of query and reference labels) between spectra of the same FLOPP-e and FLOPP identity. In contrast to the NN, the distribution of positive and negative matches for PCC shows higher overlap, which indicates that many FLOPP-e spectra have high HQI scores relative to incorrect labels (Figure S23). Therefore, when using the more efficient NN, one is less likely to encounter a mismatched classification returned in the prediction set than when using PCC.

Class-Conditional Conformal Prediction Leads to an Increase in Mean Prediction Set Size for Both Similarity Metrics. Class-conditional CP builds a unique HQI distribution for each label. CC−CP is particularly useful when the distributions of positive matches between sample and reference spectra differ among the spectral labels. In the case of the five labels used in our analysis, we noted that the HQI distribution of PP is distinct from the other labels when treated with PCC, as it exhibits HQI scores of true positive matches down to 0.4 (Figure S20). We hypothesize that the difference in score distributions between labels could be due to the impacts of signal-to-noise ratio, preprocessing steps performed by DeFrond et al. in creating the library, or a greater degree of environmental aging when compared to pristine reference spectra.[19,29,51,58] As mentioned earlier, CC−CP ensures a class-balanced confidence guarantee rather than an average guaranteed confidence (Figure S21), which results in more robust predictions at the expense of slightly larger set sizes (Figure 4). Similar to traditional CP, we observe an advantage of NN over PCC with respect to the mean set size, which we relate to the efficiency of the metric.

Conformal Prediction Guarantees Confidence and Indicates Uncertainty in Environmental Plastic Spectral Matches. Despite spectral changes that may have occurred due to weathering, the manually assigned labels of 28 environmental unknowns were frequently returned in the prediction sets generated with both PCC (82%) and NN (79%), approaching the 90% confidence guarantee afforded by CP (Figure 5). As CP's guaranteed confidence relies on an assumption that the HQI distributions of the calibration and test sets be similar, we can expect the empirical confidence to fluctuate from the theoretical guarantee when the distributions differ, perhaps due to different instrumentation used between this study and that of DeFrond et al. (see Supporting Information section IV).[44] On average, PCC returned more than one label in the prediction set, and NN returned a single correct label. NN, as the more efficient metric, better describes the inherent uncertainty in the environmental spectra. In this vein, the proportion of prediction sets with one or zero labels is an important indicator of metric efficiency and prediction quality (see Supporting Information section VII). We further used the environmental spectra to compare the traditional CP method's performance to that of CC−CP and KnowItAll and found that traditional CP outperformed both alternative methods. While the proprietary matching algorithm of KnowItAll limits our understanding of its performance, we attribute the lower performance of CC−CP to discrepancies between the distributions of label-specific test and calibration sets (see Supporting Information, section IV).

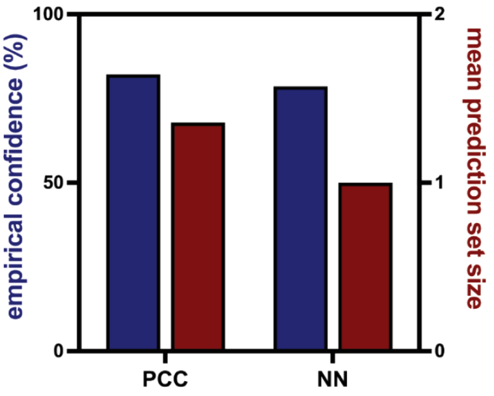


Figure 5. Empirical confidence and mean prediction set size results of conformal prediction applied to 28 environmental plastics using PCC and NN similarity metrics.

Comparing the Performance of KnowItAll and the Conformal Prediction Framework. *Conformal Prediction Reduces the Mean Prediction Set Size and Guarantees Confidence When Compared to KnowItAll.* To showcase the advantage of using CP in routine environmental microplastic identification, we compared the matching of FLOPP-e PE and PP spectra to all labels of FLOPP spectra with both KnowItAll's default correlation metric and CP using PCC and NN metrics. While the analysis pipeline of KnowItAll's SearchIt function does not guarantee confidence in spectral identification, we chose to view the results through a conformal prediction lens. To do so, we equated the proportion of instances that KnowItAll returned the correct label above an HQI threshold of 0.7 for a query spectrum with the empirical confidence associated with a prediction set generated with CP. Thus, KnowItAll's "empirical confidence" was defined as the proportion of query spectra per polymer label (FLOPP-e PE and PP) that were matched to a FLOPP spectrum of the correct label with an HQI $\geq$ 0.7. The empirical confidence of KnowItAll (~94% for PE and ~82% for PP) was used to inform the confidence guarantee used to compare CP methods (94% [α = 0.06] for PE and 82% [α = 0.18] for PP), such that the differences in the mean prediction set size were highlighted. As opposed to the mean set size for KnowItAll, here defined as the average number of spectral labels returned with HQI $\geq$ 0.7 per query spectrum, the use of CP with PCC or NN similarity metrics decreases the number of labels returned and provides guaranteed confidence in the match (Figure 6).

The Mean Prediction Set Size is Dependent on the Similarity Metric, Polymer Identity, and Confidence Guarantee. NN outperforms PCC to give a lower mean prediction set size due to fewer instances of negative matches above the defined HQI quantile, as previously described. Between query spectra identities, the mean set size of predicted labels for PP is lower than that for PE. The HQI scores to reference polymer spectra differ between the FLOPP-e PE and PP spectra, with PE having higher PCC scores to more reference labels than PP (Figures S24 and S25). This observation may explain why PE has a larger mean set size than PP, as labels with higher HQI scores are more likely to be included in the prediction set. Additionally, as the confidence guarantee for PP was reduced to reflect KnowItAll's "empirical confidence", the resultant HQI threshold is less descriptive of the true positive match score

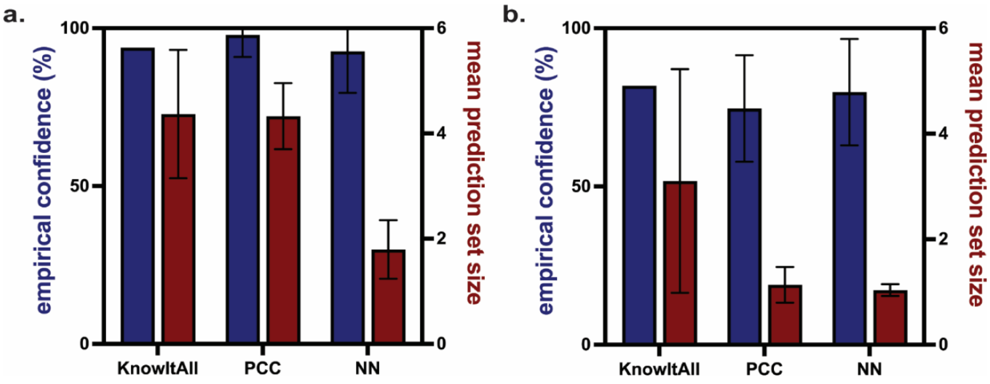


Figure 6. Results of comparison between KnowItAll and CP with PCC and NN similarity metrics in terms of empirical confidence and mean prediction set size for a) PE and b) PP.

distribution. The higher HQI score associated with a lower guaranteed confidence could limit the number of labels added to the prediction set. Across both polymer identities and magnitudes of confidence guarantees, CP methods provided statistical assurance in the spectral labeling and reduced the number of labels returned, streamlining manual interpretation of numerous matches.

Takeaways for the Microplastic Community. *The Threshold Used for Labeling an Unknown Spectrum Relies on User-Data, Similarity Metric, and Confidence Guarantee.* The performance of the CP is dependent on data quality. While we use the FLOPP and FLOPP-e libraries herein, it is important to note that different reference libraries and calibration sets may be used with the CP workflow, but the similarity metric and data analysis routine should remain consistent. As previously noted, the HQI between a reference and query spectrum can be similar for multiple reference labels (Figures S24 and S25). Therefore, it is also important that a chosen HQI threshold has a statistical guarantee of differentiation between spectral signatures. As shown in Figure 7, when using conformal prediction and PCC with all 11 labels included in both the FLOPP and FLOPP-e libraries, the confidence guarantee that the user selects has implications on both the HQI threshold and the mean prediction set size. If one opts for higher guaranteed confidence in the generated labels, the HQI quantile will be more descriptive of the data and outliers (i.e., lower), and there will be more predicted labels in the set. Therefore, choosing too high of a confidence guarantee could render the prediction sets ineffective unless subjected to tedious manual evaluation of the matches, and it may lead to false positives, causing an overestimation of MP abundance. On the other hand, choosing too low of a confidence guarantee is accompanied by a higher HQI cutoff and fewer labels in the prediction set. Here, false negatives could occur because the environmental spectra are too dissimilar from reference spectra, leading to an underestimation of MP contamination. Given this trade-off, researchers must decide the confidence guarantee with which they are comfortable when considering the balance of HQI threshold, prediction set size, and the possibility of miscounting MP quantities. Our suggestion is to choose a guaranteed confidence that is both descriptive of most of the calibration set's HQI distribution (e.g., > 70% [$\alpha < 0.3$]) and returns a mean prediction set size for manageable manual evaluation (e.g., 1−3 labels). Using Figure 7 as a guide, we chose a guaranteed confidence between 70 and 90%. While the results in Figure 7 are specific to the metrics and data discussed herein, it carries that choosing a single HQI threshold without statistical meaning and applying it to diverse environmental campaigns, as is popular in the wider MP literature, may impact the validity of one's results.

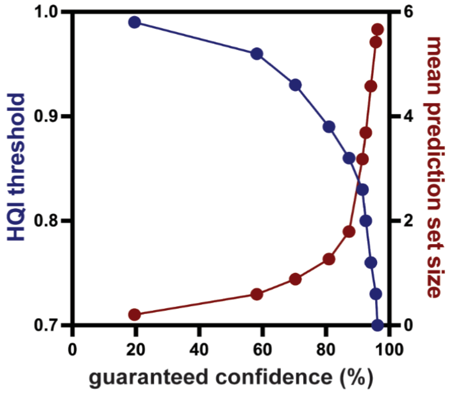


Figure 7. Hit quality index threshold and mean prediction set size as a function of the user-defined guaranteed confidence for FLOPP and FLOPP-e data using conformal prediction and PCC with 11 reference labels.

Using the Open-Access Code Available on GitHub, a Conformal Prediction Workflow Can be Used to Reduce Manual Spectral Comparison and Increase Confidence in Environmental Microplastic Labeling. Herein, we outlined a flexible framework for statistically guaranteed confidence in environmental microplastic spectral database searching. Based on our findings from a literature review of recent microplastic data analysis routines and evaluating a commercial database matching software, we illustrated the need for guidance on similarity metric comparison, hit quality index thresholding, and confidence in spectral labeling. To fill this gap, we highlighted that conformal prediction streamlines environmental plastic identification with an associated statistical uncertainty measurement and guaranteed confidence. To assist other microplastic researchers in using conformal prediction, we have generated an open-access code, user-guide, and suggested workflow (see Supporting Information section VIII) that outlines an adaptable analytical framework for statistically robust spectral labeling. With these tools in hand, we urge the microplastic community to adopt conformal prediction to meet their analytical goals for confidence in hit quality index thresholding and acceptance of environmental microplastic spectral matches.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c05167.

Additional details on methodology and literature search, similarity metrics, and conformal prediction (PDF)

User guide for open-access conformal prediction code (PDF)

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■ ABBREVIATIONS

Confidence, probability that the prediction set contains the correct identity of the unknown spectrum; Conformal prediction, machine-learning framework for uncertainty quantification; Efficiency, measure of a similarity metric's ability to depict the uncertainty of a spectrum; Hit quality index, normalized score of spectral similarity returned by the similarity metric; Label, specific chemical identity in a reference library; Prediction set, group of labels returned by the conformal prediction method; Prediction set size, number of labels returned by the conformal prediction method; Similarity metric, algorithm to compute spectral likeness via a hit quality index; Uncertainty, measure of ambiguity in spectral matches informed by the prediction set size

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